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HAIR GROWTH AND HAIR REGENERATION

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Conferences are also held at irregular intervals at times announced by special programs.

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HAIR GROWTH AND HAIR REGENERATION*

CONTENTS

* This series of papers is the result of a conference on *Hair Growth and Hair Regeneration* held by The New York Academy of Sciences on January 31, 1959.

INTRODUCTORY REMARKS

Irwin I. Lubowe
New York Medical College, New York, N. Y.

Progress in biology, medicine, nuclear medicine, physiology, genetics, and physics is proceeding at a rapid pace, and the impact and interdependence of developments in these areas will add knowledge to the investigations discussed in this monograph.

The basic concepts of the influence of hormones, steroids, enzyme systems, trace minerals such as copper and zinc, amino acids, protein metabolism, electrolyte imbalance, and ion exchange have originated and are further developed in the research laboratory. These findings are then projected into the clinical field and, after diligent study, application, and evaluation, result in the establishment of new horizons.

Several years ago the clinical course of alopecia areata and alopecia totalis was unaffected therapeutically. However, with the recent introduction of adrenocorticotropin and of the corticosteroids, cortisone, hydrocortisone and its analogues, prednisone, prednisolone, and methyl prednisolone in the medical armentarium, the outcome of these diseases can be influenced favorably. Hirsutism in the female, when related to adrenal gland dysfunction, in a significant number of cases can be controlled by the corticosteroids.

The biologist, anatomist, and medical researcher are collaborating with the clinician to establish basic findings in reference to hair loss, growth, and regeneration.

With the new evidence relative to metabolites, pigment formation, hormonal control, and histochemical and biochemical application, a more complete understanding of the behavior of hair growth, loss, and regeneration will be achieved. With the improvement and addition of new laboratory aids and techniques such as the electron microscope, chromatograph, spectrophotometer, and potentiometer, unknown vistas are being opened and mysteries clarified.

Additional knowledge is being accumulated from the investigative use of radioactive tagged sulfur, cysteine, methionine, related amino acids, and trace minerals.

INTRODUCTION

William Montagna
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The fact that the conference on which this monograph is based was the third major conference on hair growth held in nine years is evidence of the fact that this subject has come into its own. An earlier publication, *The Growth, Replacement, and Types of Hair*,[1] resulted from the first of these conferences; the second conference, *The Biology of Hair Growth* was held in 1957 at the Royal College of Surgeons in London, England. Each of the previous conferences discussed a variety of aspects of growth, differentiation, anatomy, and chemistry of hair but, because of lack of time, it was necessary to omit many things. Therefore, the principal scope of this monograph is to present material not covered by the others, to supplement the material covered by them, and to report on new findings. Naturally, it is not possible absolutely to avoid some redundancy, but an effort has been made to keep reduplication of material to a minimum. Since many odds and ends are discussed, the program is somewhat heterogeneous, lacking both logical progression and continuity. This publication rounds out the over-all picture of the state of our knowledge, fills in some details, and redefines and points up the major problems.

Newer interpretations are presented here of the patterns of hair growth in the mouse and the rat. H. B. Chase will show that these patterns are not as simple as they are believed to be. The precise patterns described earlier by himself and by others apply only to the first three coats of hair of young animals.

Advances have been made on all anatomical fronts. G. E. Rogers adds another chapter to the growing body of knowledge of the ultrastructure of hair follicles. Let one remember, however, that we have just begun to explore the fine structure of hair follicles. The electron microscopist has confined himself to growing follicles, and we know nothing about the fine structure of the follicle cells during catagen and during telogen.

Other than in the vibrissae, little attention has been paid in the past to the past to the pattern of innervation of hair follicles, yet each follicle is surrounded by nets of sensory nerves that probably subserve touch as well as other cutaneous sensations. Much of the credit for the recent advances in our understanding of the nerve endings around hair follicles must go to R. K. Winkelmann, who has pursued these studies more extensively than anyone else. Winkelmann's elegant demonstrations of these nerves and his interpretation of their significance is an important contribution to cutaneous biology.

The chemical analyses of hair roots by Rogers present still another hopeful trend in the investigation of the phenomena of growth. This is the more significant because Rogers, an excellent morphologist, gives us a much needed correlation between anatomy and chemistry. Rogers' contributions to this monograph and to cutaneous biology in general are models of excellence. Progress will be made neither by anatomical nor by chemical studies alone, but by the two combined.

In considering the problem of neogenesis of hair follicles in the adult mam-

mal, the discussions center around three main points: (1) C. Breedis and later Billingham have observed, in repairing wounds in the skin of the rabbit, that hair follicles appear to be of new formation; (2) A. M. Kligman and J. S. Strauss observed that after dermabrasion of the face in human beings, vellus hair follicles regrow, which also seem to be of new formation; and finally (3), Billingham reminds us that enormous amounts of skin and hair are formed anew each year by the cervids over the growing antlers. Of the three, however, only the skin of the antlers seems to give unequivocal proof of neoformation. Straile, who has reinvestigated neoformation in the skin of rabbits, comes to a different interpretation from that of Breedis and Billingham and, although he does not necessarily negate their results, he puts them in considerable doubt. It is inescapable, from Straile's observations, that, if neoformation does occur in the skin of the rabbit, it has not been proved. The problem of neoformation in human skin is burdened with even greater misgivings. It appears to me that, even after deep planing of the face, as Kligman and Strauss have done, fragments of hair follicles can and do remain in the skin; some of these surviving fragments can later reorganize themselves and grow a hair, and the follicles are not of new formation. Neoformation is spoken of too glibly, even by those who know better; a more thorough understanding of the anatomy of quiescent follicles could eliminate confusion.

A large part of this publication is devoted to the problems of alopecias. We have included this subject with some trepidation, since there is so little about it that we really understand. In the study of baldness, only J. B. Hamilton has made sound observations in the recent past; there has been to much talk and speculation and too little work. Many of the known basic facts about male-pattern alopecia are not known even to the dermatologist. The first step in understanding male-pattern baldness is to stop thinking about it as a degenerative, pathological state, and define it in anatomical terms. The belief that the bald scalp is devoid of hair is false. With ageing many of the follicles may degenerate as many organs do, but even the apparently naked scalp has numerous follicles, so small that they produce colorless, practically invisible hairs. Baldness is not a degenerative phenomenon, but a system of progressive changes in the entire scalp. What happens to the hair follicles is a retrograde type of metamorphosis during which the follicles become very small.

In groping for leads that might help us to understand baldness, we may study it in any experimental animal, even in mynas, as Hamilton has. Let one not confuse this seasonal occurrence with baldness; however engaging and illuminating this phenomenon may be, ultimately we must study baldness in man, who is an available and willing experimental animal. It is to be hoped that those who study baldness in the future will, regardless of their approach, keep in mind the anatomy of the scalp and the basic facts of hair growth which R. A. Ellis has expounded in this monograph.

It is unfortunate that baldness has been approached with an eye toward "regrowing" or "restoring" hair, and thus with a tendency toward commercialism. Locked within the metamorphosing hair follicles in the balding scalp are all the secrets of growth and differentiation. Searching for these secrets should transcend the eagerness to "regrow" hair on a bald scalp, an achieve-

ment which is of no great consequence. When we know these answers, we shall have the key, not to hair growth alone, but to all growth, which is, after all, the basis of all biological phenomena.

These are the beginnings of a new era of investigation into the biology of skin. The advent of new young talent into this field augurs progress. These younger investigators are bringing vigor, imagination, and new light into dermatology. Some of them are here today. Their continued efforts will surely clarify many of the riddles that still surround the growth and differentiation of skin appendages.

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Part I. Hair Growth and Development

THE GROWTH OF HAIR FOLLICLES IN WAVES*

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Waves of hair growth occur in several mammals, such as the mouse, rat, hamster, chinchilla, and rabbit, but do not occur as regular features in some other mammals: for example, the guinea pig and man. A wave may be defined as an orderly progression in time and space of follicles entering the growth phase, that is, anagen, of their cycles. A nonwave situation exists either when all follicles enter into activity simultaneously or, conversely, when follicles enter into this phase individually and without any synchronism or relationship with neighboring follicles. The mosaic type of hair replacement in the guinea pig is an example of the latter situation (Chase, 1954), each follicle proceeding through its growth cycle generations independently and with its own frequency.

The presence of a wave, that is, the orderly spreading of the skin area containing follicular activity, indicates a correlation of follicles, due either to a common and spreading initiator acting on the follicles or to an action by some means of growing follicles on their resting neighbors. Synchronism of activity can also occur without the phenomenon of a wave, as in nonspreading islands of hair growth in older mice, indicating the correlation and interdependence of follicles, but an increasing refractoriness or inability of resting follicles to respond to the presence of their growing neighbors.

The wave phenomenon is then an intermediate situation between the presence of an island of simultaneous activity and the occurrence of simultaneous activity over the whole animal. The rapidity of a wave is a reflection of the number of resting follicles ahead of the advancing edge that become active per unit time. An area of activity may have part of its periphery extending rapidly and another part not advancing at all. Two waves, especially from opposite sides, may coalesce and then proceed anteriorly or posteriorly, or both. Some areas remain quiescent (lag areas), that is, do not become involved in a wave, for one generation, but may be responsive and participate at the time of the next hair generation wave.

Reported in this paper will be the observations on the repeated spontaneous hair growth waves in colored male and female mice of different strains, ages, and physiological conditions. The complete pelage was kept carefully clipped with electric clippers, the progress of waves being observed by the presence of color due to active hair bulbs in the skin. The outlines of these areas were drawn at intervals of two days or less. Also reported and summarized in this paper will be the results of inducing hair growth by the plucking of resting hairs from different areas at different ages and the plucking of areas of different sizes.

A total of 230 mice of strains C57 BL, C57 BR, DBA, and C3H were observed

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for at least 5 hair generations. The first generation occurs as the follicles develop from the epidermis, and the order of development is from the dorsum toward the venter and extremities. This is scarcely to be considered a wave. The first replacement generation, that is, G II, proceeds generally as a wave from the venter dorsally and posteriorly (Dry, 1926). G III, however, proceeds generally from the anterior venter posteriorly, from the posterior venter anteriorly and dorsally, and then anteriorly on the dorsum after the two posterior lateral portions coalesce. G IV essentially duplicates G III on the venter, but on the dorsum there is a new center of activity on the saddle that proceeds posteriorly. From G III on, the dorsal spread from the venter stops at the lateral line, except usually in the posterior region. By G V the waves are weak in the males and, instead of waves, there are islands of growth, especially on the dorsum. In females this breakdown of waves generally occurs by G VI. Although these general patterns may represent a plurality, there are many variations, some patterns being more frequent in one strain than in another, more frequent in males than in females, and more asymmetrical in some individuals than in others. Around the head and legs there are even more extensive individual variations.

Lag areas are of particular interest in that they are regions that fail to participate in the wave of one generation, but may participate in the following wave. As early as G II such areas occasionally occur: chest, collar, base of tail, and vertex of head. In contrast to lag areas there are areas that participate each time there is a wave, the most pronounced of these being the areas at the ventral throat whorls (cowlicks), the next being the loin regions. A throat whorl might be in its tenth hair generation when a chest or base of tail region is in its fourth.

Clearly the earlier description by Dry (1926) is somewhat oversimplified, as is the more recent general statement that waves are dorsal and posterior in direction (Chase, 1954). Nevertheless, there are tendencies, and there is some order in the apparent chaos. The younger the animal, the more easily do waves "flow," that is, the less is the resistance in the hair follicles. Refractory or lag areas are those in which resistance is of relatively long but not permanent duration compared with neighboring areas. Areas such as the throat whorl, however, have very short refractory or resistant periods. The skin, in this respect at least, ages faster in males than in females and faster in some regions than in others.

Possibly in opposition to this hypothesis of resistance and presumably of an inhibitory substance (Chase, 1955), it should be mentioned that sick animals, semistarved animals, and pregnant females generally initiate no new hair growth or, at best, have occasional islands or limited waves. Here a lack of requisite nutrition for the follicles is indicated. However, "recovery" from these states results in extensive hair growth, usually of such an explosive nature that little or no wave phenomenon can be detected. In a sense the follicles were overready for going into anagen. Such an explosive or generalized growth is also known in adrenalectomized animals (Baker, 1951).

Related to the wave phenomenon is the initial synchronous growth of follicles in an area. If there is to be a wave, it proceeds from such an area, if there is

to be no wave, the area remains an island of activity. There is a critical size of responding area or a critical number of adjacent follicles. At least in the main pelage regions of the mouse, individual follicles do not enter the growth phase independently of neighboring follicles. Particularly noticeable is the observation that there are no narrow tongues of growth; even in the old animals where local islands occur, the growth includes a compact area of at least 1000 follicles, an area of at least 10 sq. mm. Such interdependence of follicles results in rounded, smooth contours of waves and islands rather than ragged contours.

Since plucking of club hairs from resting follicles is an effective method of stimulating follicle activity, areas were carefully plucked in different regions, at different ages, at different postgrowth intervals, and of different sizes. The factors so tested were all influential in the response, as might have been predicted from the above descriptions for so-called spontaneous activity. Lag areas and older animals require a larger plucked area to initiate response. The interval of time since the previous growth ceased is also important, a larger plucked area being required if the interval is short. In even the most receptive situation, there is apparently a minimum required area comprising roughly 1000 follicles. Certainly, plucking of a single hair in the mouse does not initiate anagen activity in that follicle. Mice of the obese strain were also studied. These mice homozygous for obesity, *obob*, have skin stretching to such an extent that follicles are relatively distantly separated. Plucking initiates growth and small islands do occur spontaneously, but few if any waves occur after G II, especially on the dorsum.

Particularly relevant to the plucking technique is the well-known observation that generally the plucking stimulus initiates growth, but that this growth does not spread into the adjacent unplucked region. This localization of the plucking effect has been a valid and valuable experimental technique for studying effects of various agents on growing, as compared with resting follicles. The only exception now appears to be that there will be a spread or wave if the adjacent areas are almost ready to participate in a spontaneous wave. Otherwise a plucked area on the dorsum will remain out of step with the spontaneous waves for at least one and usually more generations. Again suggesting the importance of local rather than systemic factors (Chase, 1954), the now oft-repeated experiment of Durward and Rudall (1949) should be cited. By rotating a piece of resting skin 180 degrees, the next wave will proceed in that piece of skin, except for some irregularities at the cut edges, as it would have in the original position.

Discussion

What initiates the synchronous growth activity in resting follicles and facilitates the orderly, progressive propagation of a wave? Or, from a different point of view, what prevents follicles of certain areas and at certain times from entering anagen? Large, histologically obvious vascular changes are clearly secondary responses to follicle activity and not initiatory factors, but they or lesser microcirculatory changes might be contributing factors, as will be pointed out below. The mild disturbance due to plucking or loosening of club hairs, less traumatic than a vigorous massage (Chase, 1958), certainly can induce ac-

tivity if enough hairs are plucked, but this does not directly explain spontaneous waves or islands. As yet, the most plausible explanation of the phenomena, including the chance irregularities within the general pattern regularities, is that of a more or less rapid loss or leaching of an inhibitor and a critical threshold below which anagen will occur (Chase, 1954, 1958). Neighboring activity, perhaps by way of the ancillary vascular changes, can increase the rate of loss, but not appreciably if the clubs are still firmly intact in their resting follicle capsules. As the clubs erode with time, however, very rapidly in the throat regions, the follicles lose the inhibitor built up during anagen. Whether this erosion facilitates the loss of inhibitor, as seems probable, or is merely an associated phenomenon is not known. In animals such as the regular mouse, hamster, and rabbit, where follicles are very close, especially in the young, but not so much in the obese mouse and especially the guinea pig, where distances between follicles are much greater, there is a sharing of the inhibitor. Consequently, the critical threshold at which the growth pressure or tendency becomes manifest is not reached unless several hundred follicles are involved.

This speculative hypothesis concerning an inhibitor substance does not exclude the possibility of growth-stimulating substances, but the phenomena described certainly suggest a major inhibitor component.

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RESTITUTIVE GROWTH IN THE HAIR FOLLICLE OF THE RAT*

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The purpose of this investigation is to learn to what extent the hair follicle can be altered and what regenerative capacity it possesses in both the growing and resting phases of its cycle. To investigate these aspects, it is proposed to alter the follicle by plucking the hairs and by administering antimitotic agents or thallium at intervals in the phases of the cycle.

Among the facts that may be learned through plucking in the growing stage are: how much of the follicle is removed; whether the follicle will produce a new complete hair, complete the growth that has already started, or go into the resting phase; whether the plucked follicle will be accelerated and have its own independent cycle thereafter; whether the plucking will disturb the epithelial bud for the next cycle; and whether the pigment will be disturbed and be lacking thereafter. While it has been reported[1] that plucking in the resting stage induces growth sooner than normal, how this effect is induced is not understood.

Antimitotic agents are to be administered to learn: what happens when growth is interfered with at the time the bud is actively proliferating; whether the mitotic activity can be suppressed completely; whether the size of the hair will be altered; whether other parts of the follicle have potentialities of forming hair when activity in the bud is completely suppressed; and whether substances can be applied topically that will affect the bud without causing necrosis of the surrounding tissues.

When the growth of the follicle is altered by thallium acetate, such questions arise as: Is the bud suppressed? Is keratin formation disrupted as much evidence indicates?[2] Does the hair fall out because of effects on the sheath or because of the lack of a club-shaped hair? If the depilation is merely a matter of keratinization, then the follicle should grow during the entire interval of the growing stage. If the hair is in the resting stage when the thallium is administered, it should not fall out, yet rats lose many hairs. This fact indicates that, at least, the root sheaths must be affected.

There has been much evidence to show that the effect of the thallium is exerted either through binding the cysteine or blocking the enzymatic action that is necessary for the production of the hair. Experiments will be reported on this aspect of the effect of thallium.

Methods

Rats of the Long-Evans strain were used in this investigation. In the plucking experiments, hairs were plucked from a large area of the dorsum of different rats whose hair coat was either in the growing or resting phases of the cycle. The hairs were plucked and mounted in balsam; at the same time, a biopsy was taken for correlation of the damage to the follicle. Biopsies were then

* The work described in this paper was supported by a grant from John H. Breck, Inc., Springfield, Mass.

taken at intervals following the plucking from the dorsum to determine the regrowth and repair in the follicle.

The antimitotic agent to the extent of 0.05 mg. in 0.1 cc. of water proved to be a very effective dose for subcutaneous injection, and it caused little if any irritation. Applications of solutions of 20 mg. either in 2 cc. chloral hydrate or 2 cc. salicylic acid were also often used. Topical treatment of a similar amount in 2 cc. of H_2O was also investigated.

Rats ranging in age from 1 to 40 days were given subcutaneous injections of thallium acetate. A single injection of 0.3 mg. was sufficient to produce effects in the younger rats, while 2 or 3 injections of 1 mg. at 2- or 3-day intervals were effective in the older animals. In an attempt to offset the toxic effects of the thallium in some experiments, L-cystine was injected subcutaneously or applied to the dorsum at the same time that the thallium was administered. The cystine was dissolved in water to which a few drops of concentrated NH_4OH had been added; 0.2 mg. was usually injected each time. At intervals a biopsy of skin was taken from the mid-dorsal region and prepared histologically. Skin from litter mates that received no injections of thallium were prepared for comparison.

Observations

Plucking. When the hair had erupted sufficiently to be grasped by forceps, it was plucked. At this stage of growth the bulb is actively producing cells, and keratinization is progressing distal to the bulb. Sections of the skin in FIGURE 1*a* and *b* show the extent of damage to the follicle. As may be seen, the sides of the bulb remain, while the rest of the matrix is extracted. Most of the inner root sheath with its cuticle remains intact. The matrix continues its proliferation of cells and, within 24 hours, there is a great repair and recovery (FIGURE 1*c* and *d*). Not every follicle recovers, as shown in FIGURE 1*e*. In this figure most of the follicles have recovered in 2 days, but an adjoining follicle has become spherical in shape and will finally degenerate. Many of the fine hairs of the rat's coat are difficult to pluck, and they will continue to erupt from the surface of the epidermis. FIGURE 1*f* shows the recovery that may be expected 3 days after plucking.

When growth of the hair is farther along at the time of plucking or at about the stage when pigment is no longer being produced in the hair, the hair will be broken off at the level where keratinization is taking place, as shown in FIGURE 1*g* and *h*. In this instance, of course, the hair bulb continues to produce grey or whitish hair during the growth phase, and hairs of various lengths will soon become evident on the skin surface. As the time of resting is approached there may be little or no attempt at recovery after plucking. Plucking the hair before the growth phase of the cycle had been completed neither retarded nor accelerated the time of the next growth.

When hairs are plucked in the resting phase, there appears to be little damage to the follicle other than a hyperemic condition (FIGURE 1*i*). Engorged vessels may be seen in FIGURE 1*i* near the panniculus. This hyperemic condition or some other factor immediately induces growth or activates the hair bud in some way (FIGURE 1*j*). The hair buds may be activated in a similar manner by many irritants.[3]

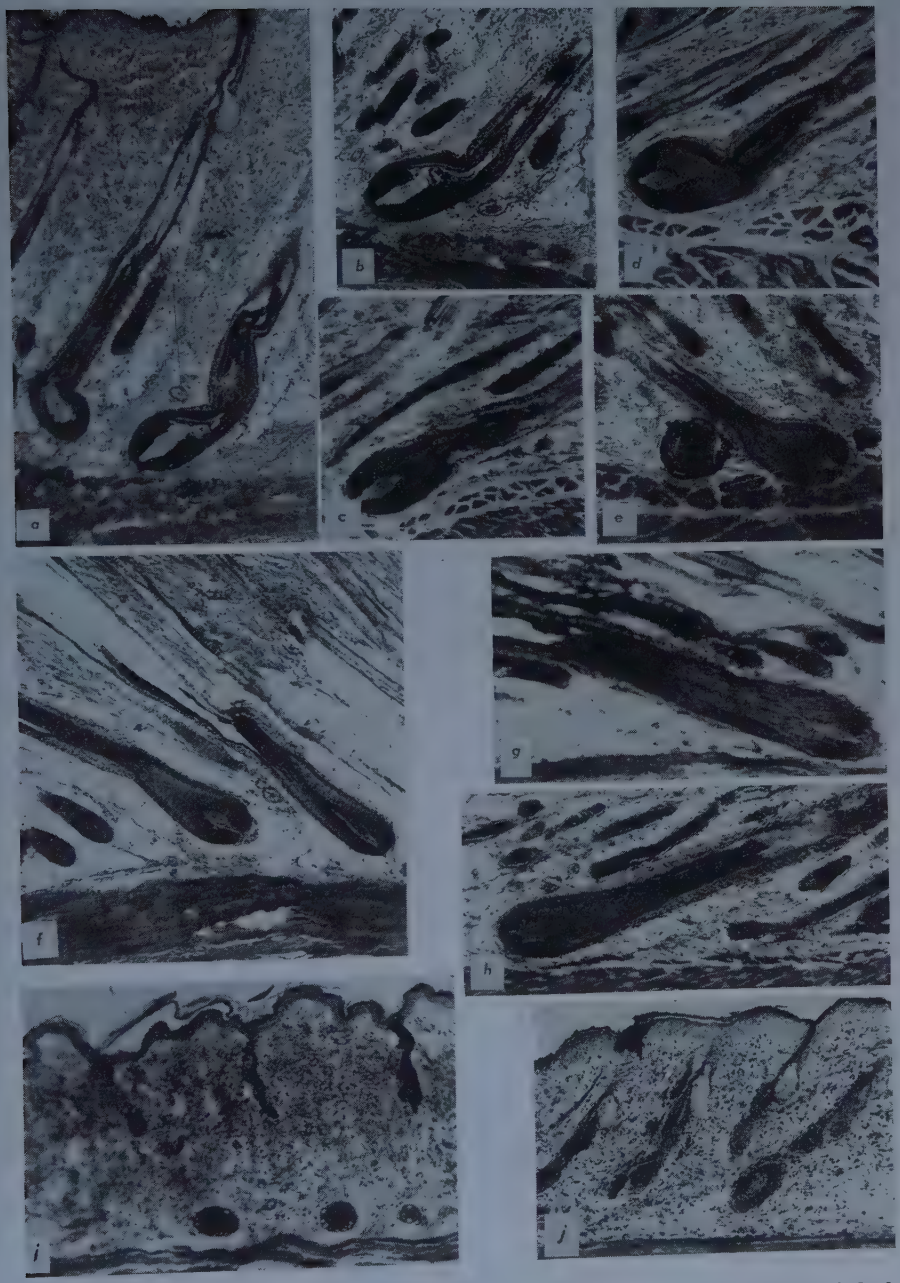

FIGURE 1*a* and *b*. The extent of damage due to plucking at the time when the hair had just erupted; (*c* and *d*) sections showing extent of recovery in 24 hours; (*e*) extent of recovery in 2 days and an adjoining degenerative spherical follicle; (*f*) extent of recovery after 3 days; (*g* and *h*) extent of damage due to plucking at the stage when pigment formation is completed; (*i*) engorged vessels that result when the hair is plucked in the resting stage; (*j*) activity of the hair bud 48 hours after plucking in the resting condition.

When the hair was in the resting phase, different areas were plucked on the same animal on the twentieth and twenty-sixth days of age. Pigment of the new hairs was apparent in the skin in the area plucked on the twenty-sixth day at the same time that pigment was visible over the rest of the back, while pigment had appeared in the area plucked on the twentieth day 4 days previously. These areas that grew hair precociously because of the plucking continued to produce hair precociously in succeeding cycles.

From these experiments it is therefore seen: (1) if the growth has not been completed, the follicle will complete the production of the hair, and injury will neither accelerate nor retard the growth; (2) plucking in the growing phase neither accelerates nor delays the time of the next growth; and (3) the time of growth can be accelerated only if hair is plucked when the follicle is in the resting condition.

Effects of the antimitotic agent. Twenty-four hours after a single injection of 0.05 mg. of the antimitotic substance, many arrested mitotic figures may be observed (FIGURE 2*a*) in the hair buds. FIGURE 2*b* shows the extent of the damage to the entire bud and, in most instances, further growth in such a follicle will not occur. In many instances where there is little mitotic activity at the time of administration, there has been much evidence that the follicle will produce a hair of fine quality.

In instances where the hair follicle is farther along in its production of hair at the time of administration, the follicles will be entirely suppressed, as shown in FIGURE 2*c* and *d*. In this instance an injection of 0.05 mg. was made and, 2 days later, a similar injection was administered. This biopsy was taken 3 days after the first injection. Seventeen days after 2 injections on successive days, skin areas such as shown in FIGURE 2*f* will be found. The follicles have been entirely eliminated. Only a few hairs will be found on the surface of such skin. In some areas of such skin new follicles are proliferating from the surface epidermis (FIGURE 2*g* and *h*). Whether or not these form from surviving epidermis or from the inward growth of epidermis from the sides of the site of injection cannot be definitely stated. Evidence is presented, however, to prove that follicles may proliferate from the surface; such proliferation has been questioned previously.

When the antimitotic agent is topically applied it is effective, as may be seen in FIGURE 2*e*. In this instance the agent was dissolved in water and was about as effective as when dissolved in chloral hydrate or salicylic acid.

The effects of the antimitotic substance, whether injected subcutaneously or applied topically, were effective chiefly in the area of administration; adjoining areas produced nearly normal follicles.

Effects of thallium. The external appearance of animals subjected to the depilatory effects of thallium has been well illustrated by Gross[4] and Thyresson.[7] While some of the histological changes in the follicle following administration have been correlated with the loss of hair, they have not been completely described.

When thallium acetate is administered to the 1-day-old animal, the first effects are most pronounced in the epidermis. Cells of the spinosum develop vacuolated cytoplasm and eccentric nuclei, and the chromatin becomes con-

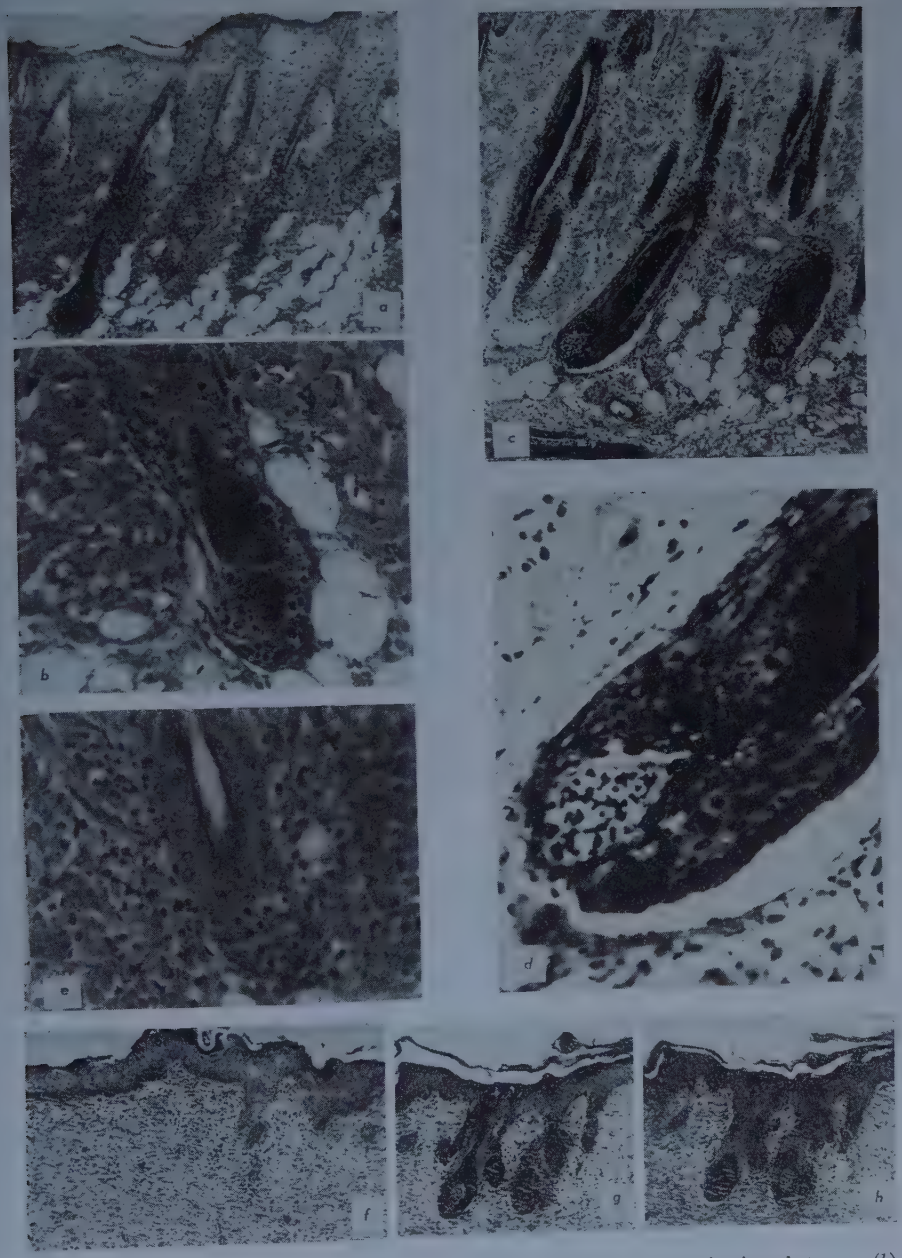

FIGURE 2*a*. Arrested mitotic figures 24 hours after injecting antimitotic substance; (*b*) section of bud greatly magnified, showing arrested mitotic figures; (*c* and *d*) effects of the antimitotic administration when growth phase had progressed; (*e*) effects of the antimitotic substance after topical applications; (*f*) skin showing the elimination of follicles 17 days after the antimitotic injections; (*g* and *h*) new buds being proliferated from surface epithelium 22 days after administration of the antimitotic substance.

densed to a pronounced pyknosis. The stratum granulosum consists of 4 or 5 layers of cells in place of the usual 2, and the cytoplasmic granules in this layer are clumped and stain very dark with hematoxylin. The lucidum is very pronounced where there is little, if any, in the normal animal, and it stains intensely with eosin. The stratum corneum is many layers thick (FIGURE 3*a* and *b*).

Keratinization progresses into the mouth of the follicle and then extends into the hair bud as it elongates and differentiates. As keratinization proceeds down the hair, vacuolization may be noted in the cells of the bud. Suppression of the multiplication of the cells of the bulb occurs, and keratinization progresses basically until nearly all cells of the hair bud are keratinized. The sheath cells likewise keratinize, and the sebaceous glands become relatively smaller. There is little left of the hair downgrowth after 6 to 8 days except a shell of cells. Little retention is supplied by the surrounding cells, and the hair is easily lost from the follicle. In the normal development of the hair a bud grows down from the epidermis, and keratin formation becomes evident just distal to the forming bulb. The hair continues to keratinize distally until it erupts through the granulosum (FIGURE 3*c*). After thallium administration, keratinization proceeds in the opposite direction or into the mouth of the follicle and then down the shaft of the hair.

When the thallium is administered at the age of 4 or 5 days, many of the hairs have begun differentiation. The keratinization of the bulb, hair shaft, and matrix above the papilla proceeds rapidly and, in a few days, only the hair in a thin shell exists, as shown in FIGURE 3*d* and *e*.

The follicle then rises, and the hair is lost if the thallium effects have diminished (FIGURE 3*f* and *g*). At the same time cells at the base of the follicle proliferate and produce a hair. FIGURE 3*h* shows follicles in the skin of a littermate that had not received thallium.

There was no success in offsetting the effects of thallium by the administration of cystine subcutaneously or topically. Either the technique was faulty or enough cystine was not administered.

Thyresson[2] claims that the hair keratin formed in this process differs from the normal in that it is a reddish mass when stained with eosin, is not yellow, and is markedly refractive. This does not appear true for, in the normal follicle, the keratin stains reddish with eosin immediately above the bulb. Keratin produced after the administration of thallium fluoresces almost as much as keratin in the normal follicles. It shows no coloration with nitroprusside and, with the Barnnett Seligman method, indicates by test for disulfide. There is every indication that such a hair is a true keratinized product.

The effects of thallium administration can be particularly well observed in the black areas of the Long-Evans rats. If the hair is removed from the dorsum when the animals are about 28 days old, then pigment will begin to appear deep in the skin 4 or 5 days later, indicating that the hair buds are becoming active. One mg. of thallium is now injected subcutaneously and, 2 days later, an additional 1 mg. administered. About 5 days after the first injection, the depilated area will be covered with short hairs that fall out in 4 days, and the area becomes completely nude. In approximately 5 days, or

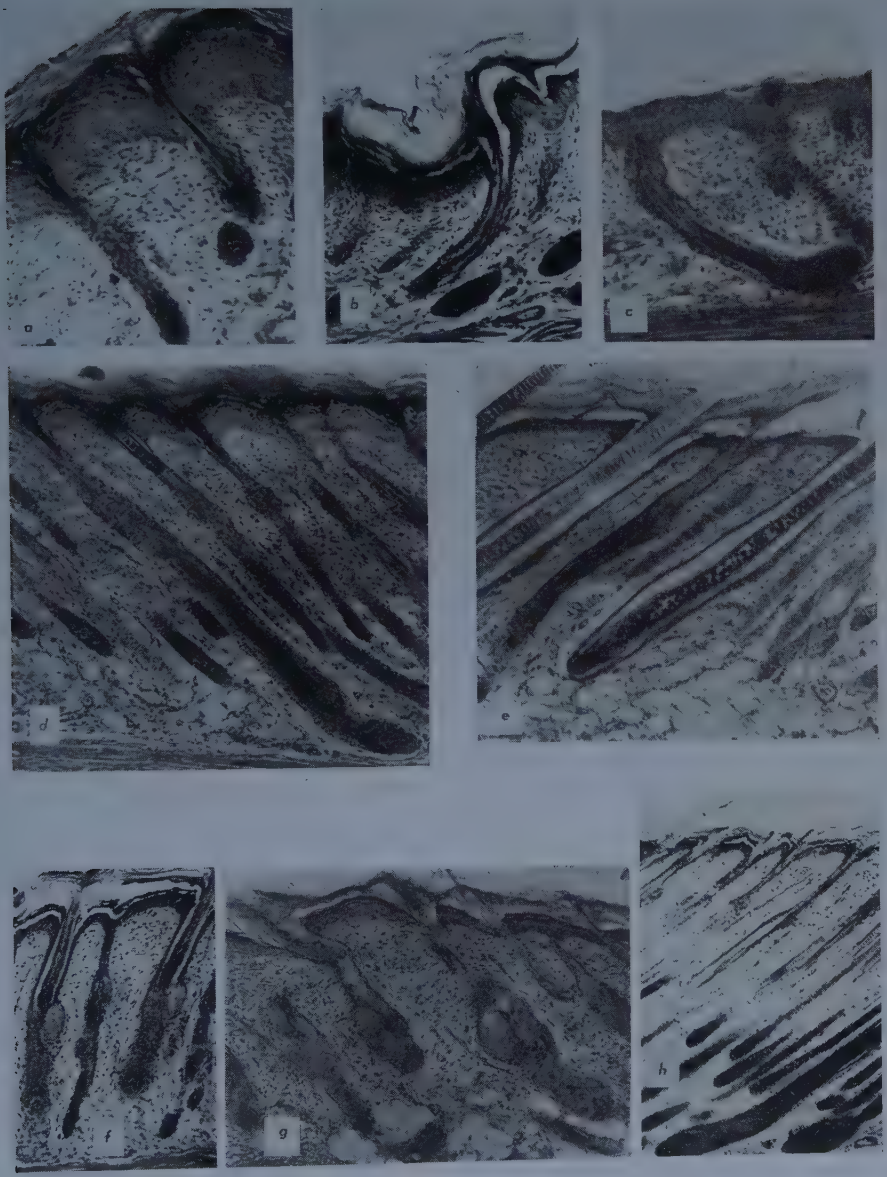

FIGURE 3*a* and *b*. The effects in a 5-day-old animal injected with thallium on its first day of life; (*c*) skin of 5-day-old normal rat. Note that keratinization occurs in opposite directions in *a* and *c*; (*d*) follicle 3 days after thallium administration—age 8 days. Given 0.2 mg. when 5 days old. (*e*) Follicle 5 days after administration of 0.4 mg.—age 10 days; (*f*) old hair and new growth. Age 12 days—given 0.4 mg. when 4 days old. (*g*) Hair being lost and new growth. Age 12 days—given 0.4 mg. when 5 days old; (*h*) growth in normal animal, littermate to animal in *f*.

14 days after the first injection, the skin again shows pigmentation, and the hair buds are again active. The new hair that now appears will survive, providing no further injections are given.

Discussion

All of these experiments indicate that, once the growth of the hair bud is initiated, every effort is made on the part of the follicle to progress through the growth phase. In the plucking experiments, the follicle recovered and continued to produce a partial hair during the growth period. With the antimitotic agent, a small hair was produced when the growth of many of the cells had been inhibited by the smaller amounts of the substance. When the dose was greater and the entire bud destroyed, then new hair buds were proliferated from the surface epithelium during the growth period. Likewise, with a decrease in the effectiveness of the thallium, growth began immediately.

Another interesting point brought out in this investigation is that my interpretation of the effects of thallium differs from those of other investigators. This seems to justify a short discussion.

The depilatory effects of thallium have long been known for rats,[4] mice,[5] and rabbits.[6] Thallium has been used clinically, as any text of pharmacology will describe. Not only is it absorbed with food, but it is also absorbed through the skin and distributed rapidly in the organism. It accumulates in the organs and skin, and is excreted slowly.[7] Thyresson[2] believed the main toxic effect was in those epidermal cells where a process of differentiation leading to keratin formation was occurring and there was a disruption of keratin formation. The present investigation shows that the hair does not fail to keratinize, but that there is an overkeratinization and a suppression of growth. If there were merely a disruption of keratin formation, the hair bud would continue to proliferate cells, and there would be a great accumulation of cells, which does not occur. Truffi[8] found degeneration changes in the hair bulbs and root sheaths in thallium poisoning. This investigation confirms the fact that the sheath cells are keratinized, with the result that the hair is loosely held in the follicle and is subsequently lost.

It is an established fact that, when thallium and cystine are both administered,[4] growth externally appears to proceed normally. From this observation and other data it has been suggested by previous investigators that the effect of thallium is either through binding the L-homocysteine and L-cysteine, resulting in a deficiency of cystine, or through blocking the enzymatic action that is necessary for the conversion of the cysteine into cystine. Lack of cystine, therefore, seems related to the increased keratinization and suppressed growth in the bud. The lack of growth and metabolism in the epidermis is probably the principal factor in keratinization. It has long been known that cystine is necessary for growth and that its lack or inhibition of utilization due to thallium could result in suppression of growth in the epidermis and the bud.

Another point that can be emphasized as a result of this work is the susceptibility of the epidermis and hair bud in such a short time. The epidermis and its derivative are easily and quickly affected by the toxic substance or, in reality, by a lack of nutrition in this case.

The lack of nutrition has probably induced keratinization; a suppression of growth or maturation is induced, and growth is inhibited. There is no evidence to show that one is the result of the other, but both are due to a common cause. The frequent observation in humans that dandruff is associated with the loss of hair is similar to the condition created here.

Since hair grows at any time and plucked follicles take up a new cycle, the connective tissue has within it substance for growth at all times; the cyclic phenomenon of hair growth does not depend upon the connective tissue.

What initiates growth of the hair bud is perplexing, as are other instances of growth, and this problem supplies one of our greatest challenges. Equally interesting is the question why growth terminates in the hair cycle.

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ELECTRON MICROSCOPE STUDIES OF HAIR AND WOOL

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The microfibril, a macromolecular particle approximately 60 Å in diameter and probably several microns in length, is now regarded as the fibrous unit of structure of α-keratins. These microfibrils can be visualized with the electron microscope,[1-6] and their presence and organization has also been deduced from the low-angle X-ray diagrams of α-keratins.[7] From electron microscope studies of chemically disrupted hairs and wool fibers[1,2] and of ultrathin sections of prekeratinized hairs[3,4] it has been established that the microfibrils are embedded in a nonfibrous cementing material or matrix that is probably rich in sulfur.[2,3,5,6]

During the development of the cortex, microfibrils are synthesized in the cytoplasm and aggregate loosely into bundles (FIGURE 1). Free microfibrils are not found in the cytoplasm beyond the stage when a number of centers of fibrillation have been formed, and it has been suggested[4] that further synthesis takes place directly onto these centers. Later the microfibrils are surrounded with matrix and are organized into larger aggregates or macrofibrils (FIGURE 2) that finally obliterate and replace the living cytoplasm of the cortical cells. The structure of a keratinized cortical cell is illustrated in FIGURE 3.

With the emergence of this fairly-clear picture of keratinizing cells at the fine structural level of organization, it has become of interest to investigate, with the electron microscope, the packing arrangements of the microfibrils and other structural details in these cells in the fully keratinized condition. Thus far, attention has been restricted to North American porcupine quill and different types of hair and wool.

The techniques for obtaining satisfactory ultrathin sections for the study of fully keratinized fibers have been more difficult to perfect than for softer tissues. Successful routine sectioning has been greatly facilitated however, by the introduction of Araldite (an epoxy resin) as an alternative to methacrylate as embedding medium.[8,9] Araldite has been tested over the past two years and has completely replaced methacrylate in some laboratories.[10]

Methods of Staining Keratins for Electron Microscopy

From studies utilizing low-angle X-ray diffraction[11] and supported by the results of electron microscopy,[5,6] it has been recognized that the only reagents that intensify the low-angle equatorial X-ray reflections to any degree and also enhance the contrast between microfibrils and matrix in the electron microscope are those that react with the cysteine or cystine side chains. In general, OsO_4 is the most satisfactory reagent for the preservation and staining of fine detail in biological structures for electron microscopy, and this has been found to hold also for keratins. Although its reactions with organic compounds are still rather ill-defined, it is known that OsO_4 reacts rapidly with the sulfur-containing amino acids.[12]

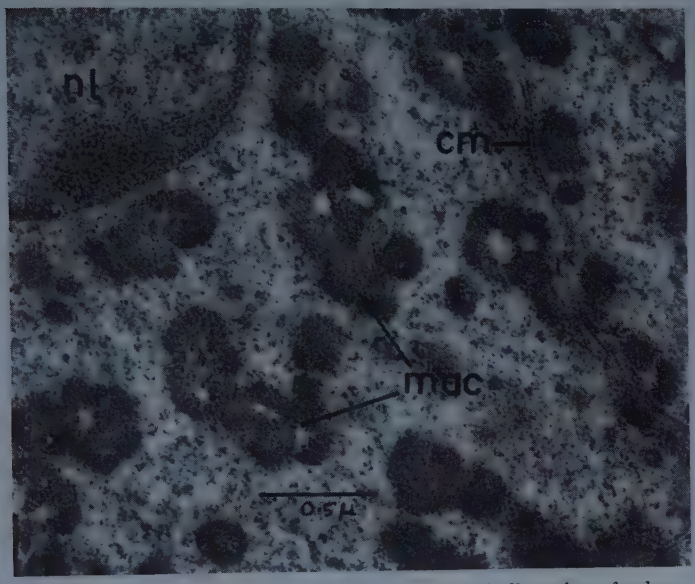

FIGURE 1. Cross section of a cortical cell in the upper bulb region of a human hair follicle. At this stage each macrofibril (*mac*) is an aggregate of dense microfibrils separated from one another by less dense spaces. Nucleus, *nl*; cell membrane, *cm*. Fixed OsO_4, *p*H 7.4.

FIGURE 2. Cross section of the fibrillation zone of a rat vibrissal follicle. In the portion of the cortical cell shown, the large macrofibril (*mac*) may be seen to consist of the microfibril + matrix complex, the matrix being the dense component between the circular profiles of the microfibrils. Fixed OsO_4, *p*H 7.4.

Of the α-keratins, porcupine-quill tip has the best resolved X-ray pattern indicating a regularity of internal structure of a high order.[13] Treatment of North American porcupine quill with OsO_4 resulted in a considerable intensification of the low-angle equatorial reflections that correspond with orders of the microfibrillar interlayer spacings[11] (FIGURE 4*a*). It is of interest therefore

FIGURE 3. Diagrammatic representation of the main structures to be found in a cortica cell (cut-away section). The oriented microfibrils (*mic*), separated by cementing matrix are bundled together into larger aggregates (*mac*). The nuclear remnant (*nl*) is in the center of the cell. The end of the cell has fingerlike processes (*i*) which interdigitate with adjacent cells.

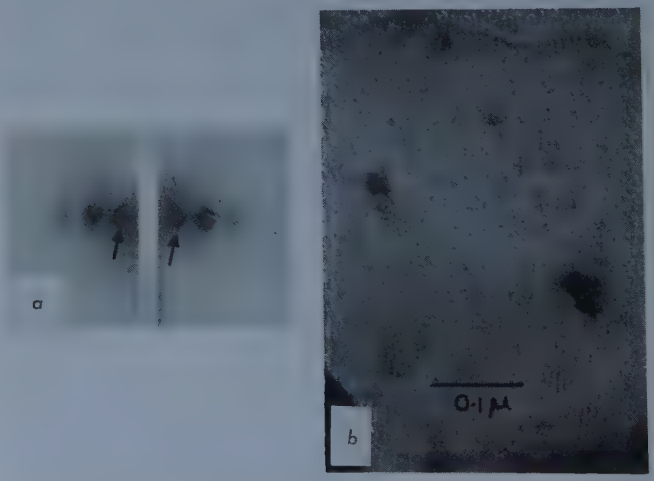

FIGURE 4*a*. X-ray pattern of North American porcupine-quill tip stained with OsO_4. Note the marked intensity of the 84 Å low-angle equatorial reflection (*arrows*). This reflection is interpreted as the fundamental interlayer spacing of the microfibril + matrix complex. (R. D. B. Fraser and T. P. MacRae, unpublished results). (*b*) Electron micrograph of a cross section of porcupine-quill tip stained with OsO_4. The microfibril + matrix complex is visible due to the deposition of osmium in the matrix.

that the "microfibril + matrix" complex, predicted by X-ray studies, can be discerned in electron micrographs of cross sections of similar specimens of porcupine quill stained only with OsO_4 (FIGURE 4*b*). The matrix between the microfibril is more electron-dense and apparently is quite accessible to OsO_4 and reacts with it. However, porcupine quill is the only keratin studied thus far in which the microfibril + matrix complex can be resolved after treatment with OsO_4 alone.

A striking increase in the clarity with which the microfibril + matrix complex can be observed with the electron microscope was found when porcupine-quill tip was treated with 0.5 M thioglycollic acid *p*H 5.6 for 24 hours,[6] washed in water and treated with 1 to 2 per cent OsO_4 for at least 72 hours. Although the over-all electron density is increased by the treatment the electron density of the matrix is increased enormously relative to that of the microfibril, so that the microfibrils become readily apparent in accurate cross sections of the tip (FIGURE 5). The contrast between the matrix and microfibrils is not as great as in some other fibers but the type of microfibril packing is characteristic. Thus within a cortical cell, groups of individually delineated microfibrils are readily distinguished (*I*, FIGURE 5). Within each of these groups the microfibrils take up various arrangements of packing, and generally this is of the type where no particular geometric arrangement prevails. However, hexagonal or near-hexagonal packing have been found (FIGURE 6*a* and *b*). These groups are separated by extensive regions where individual microfibrils are not visible and only layers of light and dense lines are to be seen (*L*, FIGURE 5). The lighter regions are microfibrillar arrays, and these layers often are oriented in several different directions in the same cortical cell (FIGURE 7).

Measurements from electron micrographs indicate that the microfibril diameters are 60 to 65 Å and the spacing between layers is about 85 Å. For the areas that show hexagonal packing the center-to-center distance is therefore of the order of 100 Å. Moreover, it should be noted that the interlayer spacing measured from electron micrographs agrees closely with the spacing indicated by the innermost equatorial reflection of the low-angle X-ray diagram[5] (FIGURE 4*a*).

The boundaries of cortical cells are separated by a distance of about 250 Å (FIGURE 5). In this gap can be seen a central dense layer (δ) about 150 Å wide, with a less dense region (β) on either side of it. Together with the cell boundaries, which also are dense, these structures constitute the cell-membrane complex that holds the cortical cells together. Extremely electron-dense regions of granular cytoplasmic debris are scattered among the extensive areas of keratin in the cortical cells (*im*, FIGURES 5 and 7). They are located between macrofibrils where fusion has not occurred, and also at the periphery of the cell. This material is more osmiophilic than the keratin even without prior treatment with thioglycollic acid. Although it is mainly protein in nature, it is different from keratin (nonkeratinous) in not being readily dissolved by alkali after oxidation with peracetic acid.

In some cross sections, small areas of cortical cells, each completely surrounded by the cell membrane complex, are found within the limits of another cortical cell (*i*, FIGURES 5 and 7). This appearance is due to the plane of sectioning passing through regions where the ends of two adjacent cortical cells

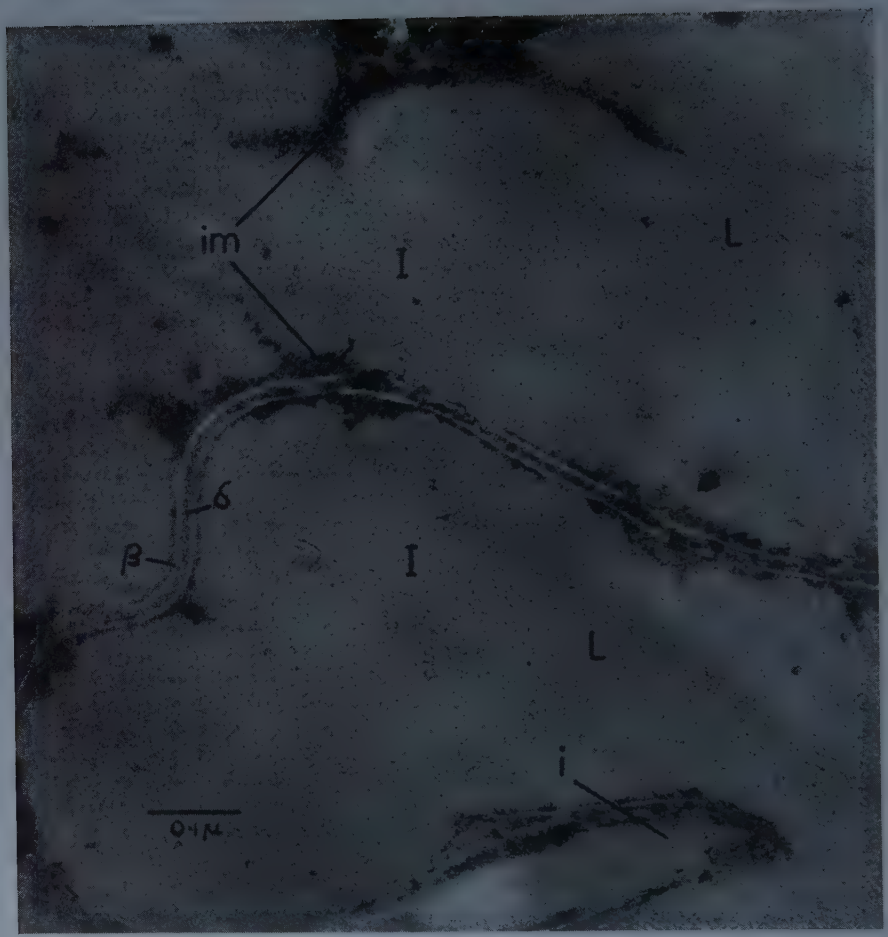

FIGURE 5. Cross section of porcupine-quill tip stained with OsO_4 after reduction with 0.5 M thioglycollic acid, *p*H 5.6 (thioglycollate—OsO_4 method). Areas of two cortical cells can be seen and, at *i*, is an interdigitating portion of a third cell completely surrounded by cell membrane complex. Note the different packing arrangements (*I*, *L*) of the microfibrils. Intermacrofibrillar material (*im*) and the intercellular (β & δ) layers of the cell membrane complex can be seen.

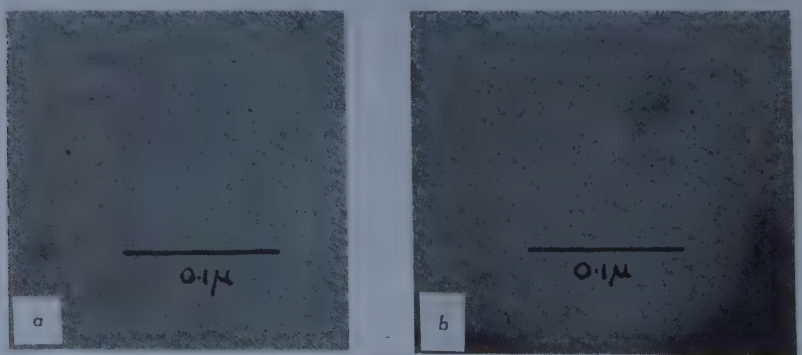

FIGURE 6*a*. Porcupine-quill tip, as in FIGURE 5, showing near-hexagonal packing of the microfibrils. (*b*) Similar to FIGURE 6*a*, but the arrangement of the microfibrils is more of the layer type. This appearance of layers may be due to tilting (with respect to the plane of section) or to irregular close packing, of the microfibrils.

interdigitate, projecting macrofibrils from one cell (*i*, FIGURE 3) residing within the other. Cortical cells isolated from porcupine quill and hairs usually have projecting macrofibrils at either or both their ends, as shown in FIGURE 8.

Some electron micrographs of porcupine quill reveal the presence of cells that are wholly or partially medullary in character (FIGURE 9). They contain

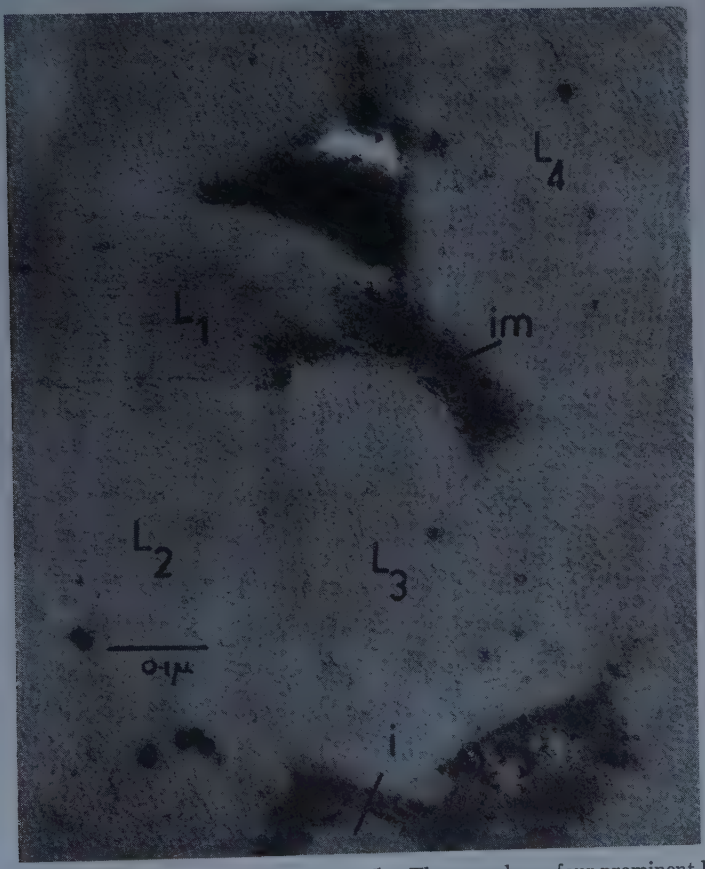

FIGURE 7. Porcupine-quill tip as in FIGURE 5. The area shows four prominent layer-type arrays of microfibrils (L_1, L_2, L_3, and L_4) oriented around the intermacrofibrillar material *im*. Groups of individual microfibrils can also be seen between some of the layers. Portion of an interdigitating cell is seen at *i*.

a high proportion of amorphous material. Pigment granules (*pi*) are also present and sectional profiles of macrofibrils from adjacent cells (*i*) project into the cells.

Microfibrillar Patterns in Hair and Wool

The microfibrillar patterns and other structural features of hair and wool discussed below bear on the problem of the bilateral structure of merino wool and the presence in many fibers of two types of cortical cell. Studies with the

FIGURE 8. A cortical cell isolated from porcupine-quill tip by treatment with trypsin. At *i* may be seen the fingerlike processes that interdigitate with adjacent cells.

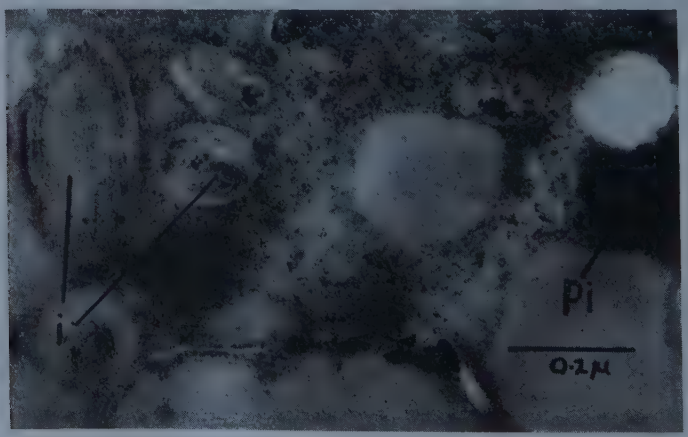

FIGURE 9. Portion of a medullary type of cell in a cross section of porcupine-quill tip stained by the thioglycollate-OsO_4 method. The cell contains quantities of amorphous material instead of keratin. Pigment granules (*pi*), and portions of other cells (*i*) surrounded by cell membrane complex are also present.

light microscope in recent years[14-17] have revealed that in fine merino wool in particular but in certain other finely-crimped wools as well, the cortex has bilateral properties. Thus there are two cortical segments that differ in their accessibility to dyes, the orthocortex dyeing more intensely and uniformly than the paracortex (FIGURE 10*a*). The paracortex, on the other hand, is more resistant to attack by reagents that swell and digest keratin.

Studies of coarser types of wool fibers after dyeing have shown these to tend to a radial symmetry of dye distribution[16,18,19,20] rather than to a bilateral one,

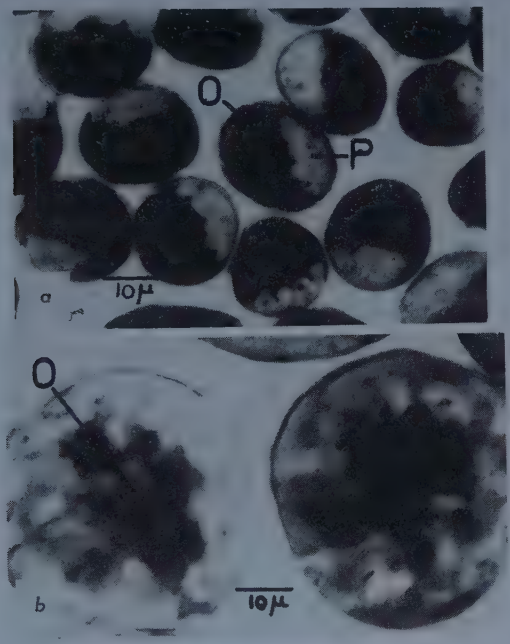

FIGURE 10*a*. Light micrograph of cross sections of merino wool fibers dyed with methylene blue to show up the bilateral structure. The densely-dyed portion is orthocortex (*O*), the other is paracortex (*P*). In the latter, cortical cell outlines and nuclear remnants may be seen. (*b*) Light micrograph of dyed Lincoln wool fibers. The densely-dyed centrally-placed cells resemble orthocortical cells, whereas the remainder appear to vary in type from transitional to more or less like those of the paracortex.

the orthocortical cells often being in the center of each fiber (FIGURE 10*b*). Again the cortical cells of some fibers vary widely in their intensity of dyeing and show no particular distribution. On the basis of dyeing and swelling experiments, some investigators have suggested that human hair is predominantly "para" and mohair largely "ortho"[20] in their properties.

Several different types of wool and hair fibers were examined in cross section by electron microscopy after treatment with thioglycollic acid, *p*H 5.6, staining with OsO_4 and embedding in Araldite. The fibers included human hair, a mutant merino wool in which the fine fibers are lacking in crimp due to a single dominant gene,[21] rabbit hair, Lincoln wool, fine merino wool, steely

merino wool—due to copper deficiency in the sheep,[22] mohair and rabbit fur. It was found that the clarity of definition of the microfibril + matrix complex, that is, the electron-density of the matrix relative to that of the microfibrils, and the frequency with which the complex could be detected in each type of fiber, decreased, insofar as could be judged, in the order in which the fibers are listed. Moreover, from the analytical figures available, indications are that the cystine contents decrease in the same order. Thus there is a qualitative correlation between the clarity of definition of the microfibrils in electron micrographs and the cystine content of the fibers.

The increases in weight of human hair and merino wool fibers were measured under the conditions of staining used in these experiments. They were 44 per cent and 36 per cent respectively. This increase could not arise solely from reaction of the OsO_4 with the cystine of the fiber keratin and it indicates that reactions with other amino acid side chains (for example, tyrosine) occur. From the electron density of the matrix it is apparent that much OsO_4 enters this component and could be expected to increase the distance between microfibrils from its value in unstained fibers. That this in fact occurs has been established from the low-angle X-ray reflections,[5] the increase being greatest in human hair with the highest sulfur content and amounting to about 8 Å.

Electron micrographs of cross sections of merino wool, stained with OsO_4 after thioglycollic acid treatment, show bilateral differences in structure at low magnifications. Thus in FIGURE 11 it is readily apparent that the organization in the paracortex and orthocortex is distinctly different. In the orthocortex approximately circular profiles of macrofibrils surrounded by dense cytoplasmic debris can be seen. In contrast the keratin of the paracortical cells has a more homogeneous appearance with comparatively little intermacrofibrillar material interrupting the extensive fusion of the component macrofibrils.

At higher magnifications the paracortex and orthocortex differ in the organization of their component microfibrils and matrix.[6] Thus the microfibril + matrix complex is usually visible in the paracortical cells, individual microfibrils being clearly delineated over large areas, groups of them being packed in near-hexagonal arrays. Other groups show less-regular packing but areas where the packing is in layers, as in porcupine quill, occur infrequently. In the orthocortex the macrofibrils have the appearance of whorls, the microfibrils being arranged in concentric layers with individual microfibrils distinguishable only in the innermost layers.[6] Frequently the contrast between the microfibrils and matrix is too low for the complex to be visible in the orthocortex.

Ortho- and paratype cells can be distinguished in other fiber types. In general it is found that in those cells which, at low magnifications, are homogeneous in appearance due to a low content of intermacrofibrillar debris (paratype), the microfibril + matrix complex is visible at higher magnifications. Cortical cells that have much intermacrofibrillar debris (orthotype) have smaller macrofibrils with a microfibril + matrix complex, if visible, of the "whorly" type. In some fibers the difference between ortho- and paratypes of cell is less distinct, these cells being transitional types.

Human hair has no bilateral structure. However, its cells show both types

of microfibrillar packing although they differ from merino wool in the invariably high degree of contrast between matrix and microfibrils. Thus in FIGURE 12 is a cell that contains much intermacrofibrillar material and macrofibrils with the appearance of whorls, thereby resembling an orthocortical cell of merino wool. In contrast, in FIGURE 13 is an area where the microfibrils are mostly individually visible and whorls are not prominent.

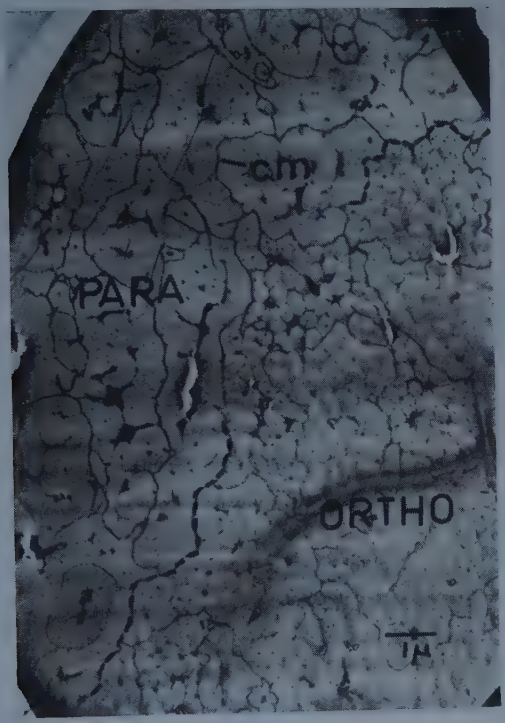

FIGURE 11. Low magnification view of a cross section of a fine crimpy merino wool fiber stained by the thioglycollate-OsO_4 method. On the left is the paracortex in which the keratin is more homogeneous in appearance than in the orthocortex (*ortho*). Approximately circular macrofibrils separated by nonkeratinous material can be seen in the orthocortical cells. The boundary between the two segments is clearly seen. Nuclear remnants are prominent in the paracortex. Cell boundaries (*cm*) are clearly defined.

In hair fibers of the rabbit, the microfibril + matrix complex is visible in cross sections of the guard hairs but only rarely in the fur fibers. The contrast between the matrix and microfibrils in the microfibrillar patterns of the hairs is similar to that in human hair and the whorly pattern is particularly frequent in occurrence (FIGURE 14).

In mutant merino wool fibers the cortical cells are homogeneous in appearance (FIGURE 15*a*) and resemble the paracortical cells of normal merino fibers (FIGURE 11). At higher magnification the microfibrils are found to be delineated (FIGURE 15*b*) almost as clearly as those in human hair.

At low magnifications Lincoln wool fibers are seen to contain cortical cells

which would fall into the two groups of ortho and para, but some of the cells are intermediate between the two main groups. At higher magnifications the microfibril + matrix complex is clearly visible in many cells (FIGURE 16). Discrete microfibrils are found over large areas of the cortex. They often occur in crystalline packets which are oriented at different angles to one another.

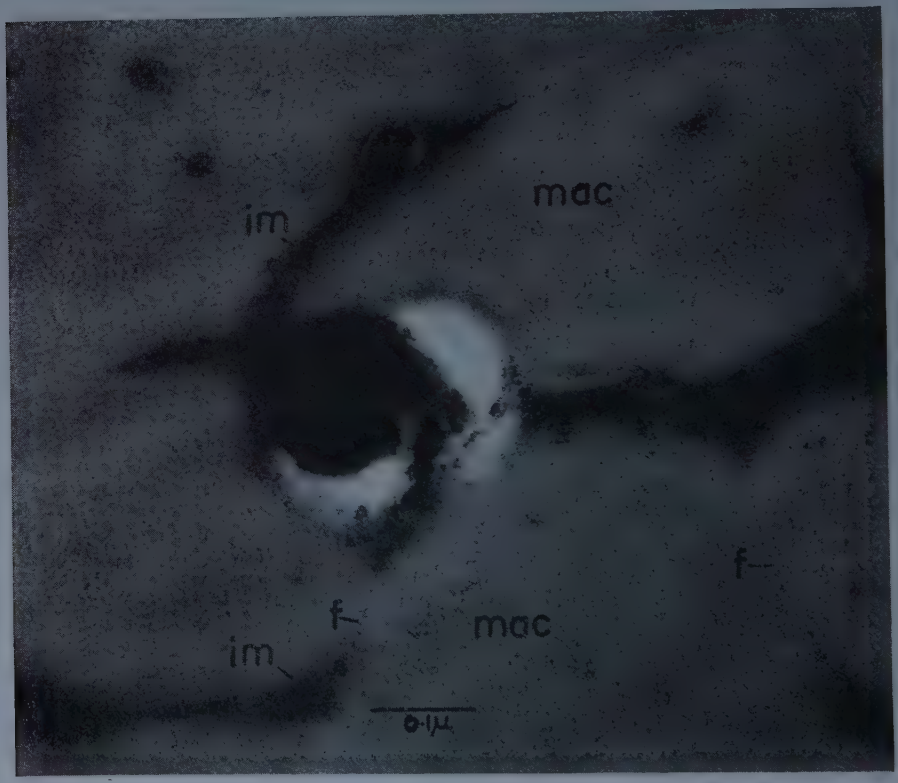

FIGURE 12. Cross section of human hair stained by the thioglycollate-OsO_4 method. The structure of the cell shown here resembles that of an orthocortical cell of merino wool. The approximately circular macrofibrils (*mac*) have the appearance of whorls, individual microfibrils being visible in their centers and surrounded by microfibrils in layers. Intermacrofibrillar material (*im*) is prevalent and the boundaries (*f*) of adjacent macrofibrils are easily discerned.

Whorly macrofibrils have been observed in some cells. In FIGURE 16 are to be seen several regions of Lincoln wool cortex where interdigitation occurs between cortical cells meeting end-to-end.

It is difficult to find the microfibril + matrix complex in mohair, the contrast being very low. Thus no fine structure has been observed in macrofibrils of the cells of the orthotype, but some is to be found in paratype cells. Steely wool is similar to mohair as regards the low contrast. However, these fibers often have a small group of paratype cells, in this way resembling the bilateral structure of normal merino wool.

Although a number of fiber types have been examined it is uncertain to what extent the OsO_4 penetrates the microfibrils; hence accurate measurements and comparisons of spacings from electron micrographs of different fibers are not possible. However, it is evident that the diameters of microfibrils in different fibers are closely similar.

There are two possible explanations of the layer structures that are prevalent in the microfibrillar patterns of North American porcupine quill and also present in hair and wool. Thus regular arrays of microfibrils might be tilted with respect to the plane of sectioning so that their projected images fuse to

FIGURE 13. As in FIGURE 12, but the cell here resembles a paracortical cell, the macrofibrils being less of the whorly type.

give the impression of layers. Alternatively, the microfibrils might be polarized physically or chemically so that they tend to form aggregates in sheets with more matrix between the sheets than between the microfibrils within each sheet.[5] Aggregates of this form have been isolated from wool.[2] Again, in cortical cells of the orthocortex, the sheets of microfibrils are disposed in concentric layers and the microfibrils are almost certainly arranged helically along the macrofibril axis.[5,6]

If the microfibrils are tilted or are helically arranged with respect to the fiber axis then it would be expected that tilting of the sections in the electron microscope would alter the appearance of the microfibrillar patterns. Discretely visible microfibrils should be replaced by a layer structure, and vice versa. Although changes of this nature have been observed, gross changes in the microfibrillar patterns have not so far been found. However, further work

is necessary in order to critically assess the effect of the thickness of the sections on such changes in the microfibrillar patterns.

The Distribution of Intermacrofibrillar Material in Different Wool Fibers

The presence of cytoplasmic debris between macrofibrils can be readily revealed if fibers are treated with 0.5 M thioglycollic acid at 50° C. for 24 to 48

FIGURE 14. Cross section of a guard-hair of the rabbit stained by the thioglycollate-OsO_4 method. The keratin of two adjacent cells can be seen separated by the intercellular layers β and δ. The microfibril + matrix complex is very clearly defined and the whorly macrofibrils (*mac*) are similar to those of the orthocortex. They are separated by dense material (*im*). The arrows indicate individually delineated microfibrils in the midst of microfibrillar layers.

hours, washed, and then stained with OsO_4. A partial extraction of the keratin proteins (30 to 50 per cent) occurs under these conditions[23] and the intermacrofibrillar material is more densely stained than the keratin remaining in the fibers. In FIGURE 17*a* the bilateral distribution of this material in a merino wool fiber can be seen. Very little is present in the paracortex, but

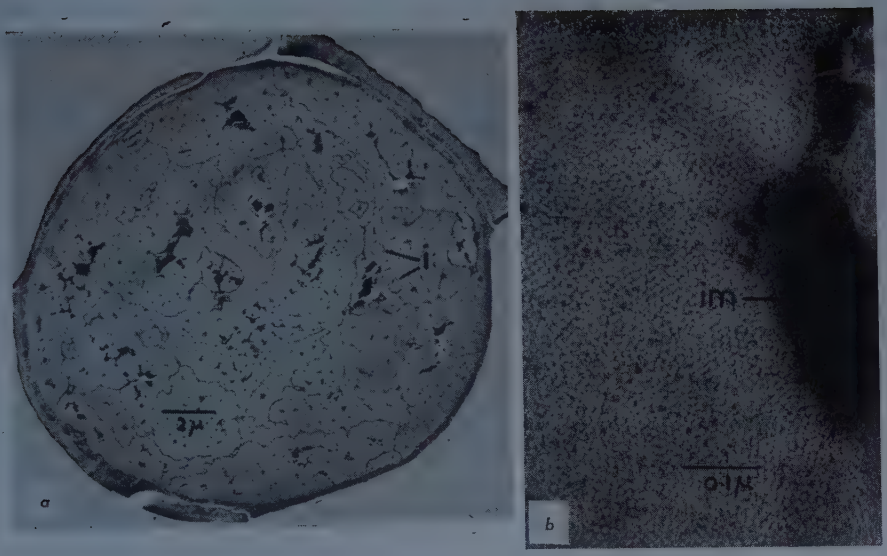

FIGURE 15*a*. Low-magnification view of a crimpless fiber of mutant merino wool, stained by the thioglycollate-OsO_4 method. The cell outlines, the homogeneous appearance of the keratin in the cells, and the prominent nuclear remnants are features that resemble those of the paracortex of a normal merino wool fiber. Interdigitating processes can also be seen (*i*). (*b*) A high-magnification view of portion of a cell in FIGURE 15*a*. The outlines of microfibrils are clearly visible due to the dense staining of the matrix as in the paracortex of a normal merino fiber. Groups of near-hexagonally packed microfibrils can be seen. Whorly macrofibrils characteristic of orthocortical cells are not present. Intermacrofibrillar material at *im*.

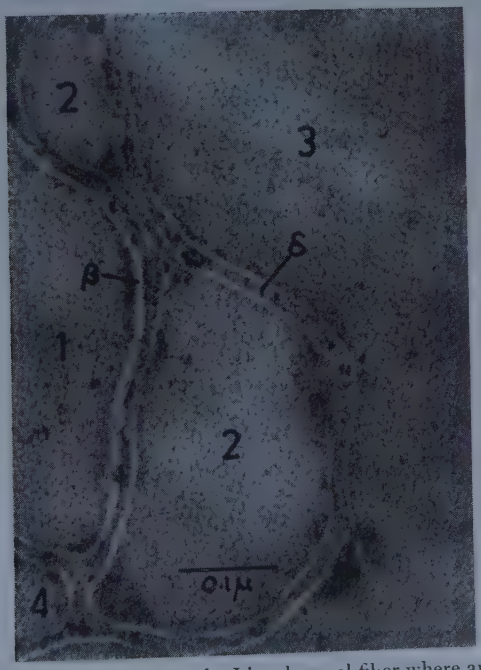

FIGURE 16. Cross section of portion of a Lincoln wool fiber where areas of several cortical cells (1, 2, 3, and 4) interlock and are separated by the intercellular layers β and δ. The microfibril + matrix complex can be seen. Whorly macrofibrils are present in Lincoln wool fibers but are not shown here.

there is an abundance of it in the orthocortex. In crimpless mutant merino wool fibers most of the cells have an appearance resembling those of the paracortex (FIGURE 17*b*), whereas the cells of kid mohair resemble those of the

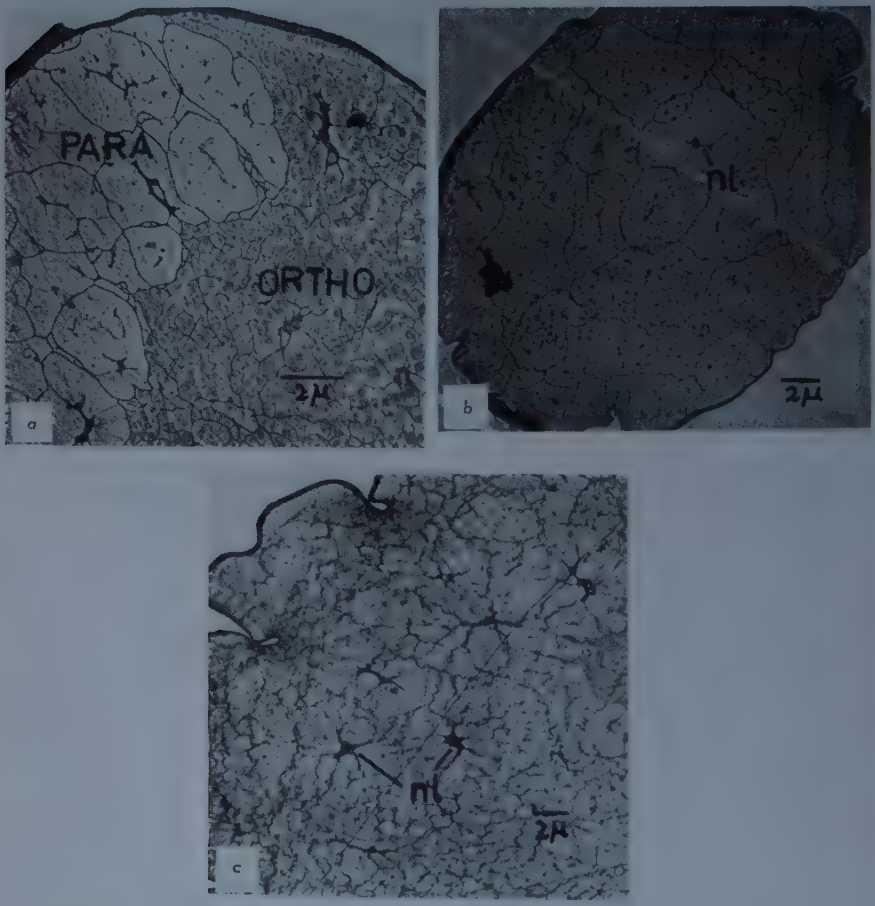

FIGURE 17*a*. Cross section of a merino wool fiber that was extracted with 0.5 M thioglycollic acid at 50° C., and then stained with OsO_4. This treatment removes portion of the keratin of the fiber and reveals the network of abundant nonkeratinous material in the orthocortex. The outlines of individual cells are masked, whereas the cells of the paracortex are clearly outlined. It can be seen that the boundary between the cortical segments is well-defined. (*b*) Cross section of a crimpless mutant merino wool fiber treated as in FIGURE 17*a*. Note how the cells resemble the paracortical cells of the merino fiber in FIGURE 17*a*. Nuclear remnant, *nl*. (*c*) Cross section of a mohair fiber treated as in FIGURE 17*a*. Intermacrofibrillar material is abundant and cell outlines cannot be distinguished. The fiber resembles the orthocortex of the merino fiber in FIGURE 17*a*. Nuclear remnant, *nl*.

orthocortex (FIGURE 17*c*). Again, the examination of cross sections of Lincoln fibers revealed the presence of many cells of transitional type. In the case of mutant merino wool and mohair it is of interest that they have been classified as paralike and ortholike respectively on the basis of their staining reactions with various dyes.[20]

There can be no doubt that the relative abundance of intermacrofibrillar debris in orthocortical cells must increase their accessibility to dyes compared with the paracortex. That this intermacrofibrillar material stains intensely has been observed by the light microscopy of cross-sections of dyed fibers.

The Question of a Cystine-Rich Matrix between Microfibrils

In order to explain the high-angle X-ray diffraction pattern of α-keratins, Astbury and Dickinson first suggested that the crystallites that give the α-diagram might have a low content of cystine but are surrounded by a cystine-rich matrix.[13] Moreover, it has been found that reaction of the cystine with heavy-metal stains does not affect the high-angle X-ray pattern.[11]

Early studies of wool by electron microscopy indeed revealed the presence of amorphous cementlike material between the fibrils,[1,24] and later work[2] indicated that it was necessary to break a proportion of disulfide bonds before microfibrillar aggregates were released from wool fibers.

Chemical studies have shown that protein fractions with high and low cystine contents can be isolated from wool[25-27] and that the low-sulfur protein is the one that is fibrous and gives an α-keratin diagram with X rays[25] (see also Rogers[28]).

More recent electron microscope studies[3,4] of developing hairs stained with OsO_4 showed in the keratogenous zone microfibrils surrounded by an electron-dense matrix (FIGURE 2). In view of the known rapid reaction of OsO_4 with —SH groups[12] this provided fairly convincing evidence that the OsO_4 had been reduced by the cysteine side-chains in the matrix.

Evidence of a direct nature was obtained in a recent electron microscope study[6] in which it was shown that a cystine-rich protein similar to γ-keratose in composition,[29] originating from between the microfibrils, could be extracted from oxidized wool. The intense electron density of the matrix produced by the thioglycollic acid-OsO_4 staining method used in the present study also indicates that the concentration of reactive disulfide bonds is higher in the matrix than in the microfibrils.

From the electron microscope studies, differences in the manifestation of the microfibril + matrix complex in different types of fibers suggest that either the amount of matrix between the microfibrils differs considerably, or alternatively, that the cystine content of the matrix itself varies. The electron microscope results do not allow a choice to be made. However, measurements from low-angle X-ray diagrams favor the former alternative, since the distance between centers of adjacent microfibrils appears to increase with increasing cystine content.[5]

A further related and important question is that of the origin of the matrix, and there would appear to be three different ways in which this component might arise between the microfibrils.

The most probable explanation is that the matrix is synthesized as such either by the cortical cell itself or even in another follicle layer, as in the outer root sheath. At a certain stage during fibrillation the matrix is located between the microfibrils where it is finally cross linked when keratinization is complete. However, it is possible that as the microfibrils are synthesized in the cell and aggregate loosely into fibrils they might become associated with a cystine-defi-

cient matrix that does not show up in electron micrographs (FIGURE 1) but is later enriched with sulfur by a reaction of its serine side chains with free methionine, the reaction known to take place in the formation of cysteine (Marston[22]). Alternatively the matrix might be derived from the microfibrils themselves by the disorganization of their peripheral protein chains, which could be enriched by the reaction referred to above.

Present evidence cannot distinguish between these alternatives, but accurate measurements of microfibril diameters could be useful here. Of possible significance is the fact that the serine content of γ-keratose (derived from the matrix) is higher than in α-keratose[29] (derived from the microfibrils).

Bilateral Structure of Merino Wool Fibers

There is considerable chemical and histochemical evidence indicating a higher cystine content in the paracortex than in the orthocortex[16,20,30] and, in respect of this quantitative difference, it is interesting that electron micrographs of the two segments have revealed a higher electron density of the matrix in the paracortex than in the orthocortex. This suggests that either there is more matrix between the microfibrils or its cystine content is higher in the paracortex.

The possible influence of the intermacrofibrillar protein on the intensity of dyeing of the orthocortex has already been mentioned. The major effect of this material, however, is probably on the rate of dye penetration into the macrofibrils of the orthocortex rather than on the final intensity of dyeing in this segment. Since the two segments are distinguishable even after dyeing to equilibrium,[20] the explanation must be in the dye-binding properties of the microfibril and the matrix. One possible explanation involves consideration of the protein fractions isolated from wool that are low and high in sulfur,[25-27] and probably derive from the microfibrils and the matrix respectively[3,6,25] (see also Rogers[28]). Quantitative amino acid analyses[27-29] of these protein fractions indicate that the microfibrils are much higher in their content of the acidic, basic, and neutral amino acids than the matrix. Thus if the ratio of matrix:microfibril were low in the orthocortex compared with the paracortex (as suggested by electron microscopy) then a predominance of acidic and basic groups in the orthocortex would follow and hence also a higher uptake of acidic and basic dyes in this region.

The presence of an abundance of intermacrofibrillar debris in orthocortical cells and the lower sulfur content of these cells[16,20,30] indicate a fundamental difference in the metabolic activity of orthocortical and paracortical cells. In accord with an earlier suggestion[16] this difference possibly originates during cellular differentiation in the follicle bulb although it may be influenced later by other follicle layers.

Fine Structure of the Microfibril

That there may be structural features within the microfibrils themselves resolvable by high-resolution electron microscopy, is indicated by electron micrographs of human hair stained with OsO_4 after reduction by thioglycollic acid at *p*H 5.6. In FIGURE 18 can be seen a group of microfibrils, several of

them having electron-dense cores about one fourth to one third of their total diameter due to the penetration of the OsO_4 into this region. Groups of similar microfibrils have also been observed in cross sections of mutant merino wool and of North American porcupine quill. Since dense cores are not found in every microfibril in a cross section it follows that the site in the microfibril that reacts with the OsO_4 might occur periodically along the microfibril and hence would not necessarily coincide with the plane of section.

At present it is not possible to correlate this feature of the microfibril with the groups of compound helices of which microfibrils are believed to be composed[31] or, alternatively, with the formation of helical aggregates from globular precursors.[32]

Longitudinal Sections

Although technically it is easier to cut cross sections of keratinized fibers, longitudinal sections of porcupine quill have been obtained suitable for study.

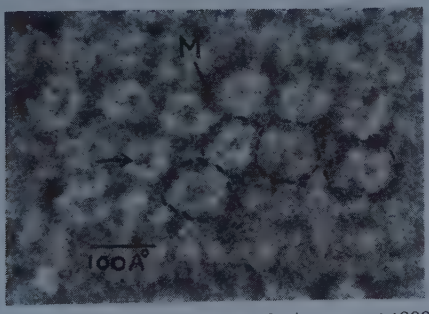

FIGURE 18. High-resolution electron micrograph (approx. ×800,000) of a cross section of a human hair stained by the thioglycollate-OsO_4 method in order to reveal the microfibril + matrix complex. The microfibrils (*outlines dotted*) are surrounded by dense matrix (M). Some of the microfibrils have dense cores (*arrow*).

A typical example is shown in FIGURE 19, in which the light and dense lines correspond with the microfibrils and matrix respectively. Despite the unlikelihood that the section plane remains within a microfibril layer it is interesting to note that the outlines of individual microfibrils have been followed for distances up to about 1 μ.

Thus far no structural regularities along the microfibril axis have been resolved after treatment with OsO_4 or I_2 (in alcohol). Although no such regularity would be expected in the case of OsO_4 in view of negative X-ray evidence, on the other hand I_2 treatment intensifies the low-angle meridional X-ray reflections corresponding to long-range periodicities.[11]

Aspects of Cuticle Structure

Knowledge of the structure of the cuticle began with early analyses of the cuticle by X rays and birefringence,[33,34] which showed that, in contrast to the cortex, the cells of the cuticle contain amorphous protein. Later work, using chiefly the electron microscope to study fragments and replicas of fibers[35,38] and cross sections of developing hair,[4,39] established the fact that there are two

intracellular layers in a cuticle cell: the outer one known as the exocuticle, the inner one as the endocuticle. When a cuticle cell is located outermost on a fiber, its outer membrane (epicuticle[36]) is found to be very resistant to chemical attack.

Studies of cross sections of developing hair[4,39] have shown that the exocuticle is formed as a well-defined layer of large amorphous aggregates below the epicuticle (FIGURE 20*a*), whereas the endocuticle appears to be formed from cytoplasmic debris, in this way resembling intermacrofibrillar material. The endocuticle is nonkeratinous whereas the exocuticle appears to be stabilized

FIGURE 19. Longitudinal section of porcupine-quill tip stained by the thioglycollate-OsO_4 method. The alternate light and dark lines running in the direction of the arrow correspond to the microfibrils and matrix respectively. Individual microfibrils can be followed for a considerable distance.

by disulfide bonds.[39] Evidence of the keratinous nature of the exocuticle was found when cross sections of wool follicles were oxidized with peracetic acid and then stained with methylene blue. This procedure stains keratin intensely[16] and, under the light microscope, the exocuticle of the developing cuticle was observed also to stain intensely, (FIGURE 20*b*).

The study of cross sections of fully keratinized fibers stained with OsO_4, has shown by electron microscopy[6] that individual cuticle cells are separated by a prominent intercellular layer (δ, FIGURE 20*c*) similar to that found between cortical cells, and submicroscopic "swellings" of this layer have been observed in certain wool fibers.[6] A further feature of interest is the interlocking of adjacent cuticle cells by well-defined projections (*i*, FIGURE 20*d*) each projection being surrounded by the cell membrane complex (FIGURE 20*d*). These structures must contribute to the rigidity and strength of the cuticle.

The outline of the boundary between the exocuticle and the endocuticle is often visible in fibers stained with OsO_4 (FIGURE 20*c*). In the endocuticle dense aggregates are found due to the looser texture of this layer.

It has been observed that the exocuticle has a greater electron density on

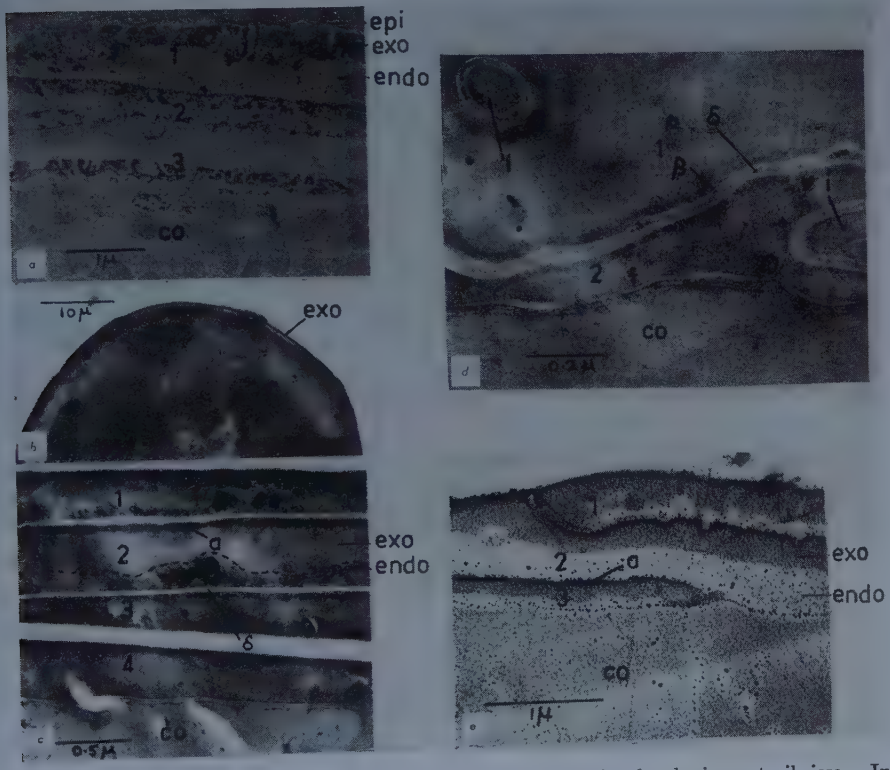

FIGURE 20*a*. Cross section of cuticle cells (1, 2, and 3) of a developing rat vibrissa. In the outermost cell (1) can be seen the epicuticle membrane (*epi*), the aggregates of amorphous protein in the exocuticle layer (*exo*) and the endocuticle layer (*endo*). Cortex (*co*) can also be seen. (*b*) Light micrograph of a cross section of a developing wool fiber oxidized with peracetic acid and stained with methylene blue. Structures with high concentrations of disulfide bonds (for example, keratin in the cortical cells) stain deeply. The exocuticle (*exo*) is densely stained and therefore is keratinous. (*c*) Four cuticle cells (1, 2, 3, and 4) and part of the cortex (*co*) of a cross section of a human hair stained by the thioglycollate-OsO_4 method. Several layers can be seen in each cuticle cell. Thus in cell 2 there is an outermost dense layer *a* in the exocuticle. The exocuticle (*exo*) is clearly defined from the endocuticle (*endo*), which has a granular appearance. Between the cells may be seen the prominent dense intercellular layer. (*d*) Two cuticle cells (1 and 2) and the cortex (*co*) of a Lincoln wool fiber are shown. The cuticle cells are separated by the intercellular layers β and δ of the cell membrane complex. Note the areas *i* surrounded by cell membrane complex. These areas are from interdigitating fingerlike processes of an adjoining cuticle cell. (*e*) Cross section of a Lincoln wool fiber reduced with thioglycollic acid *p*H 5.6, and coupled with methyl mercuric iodide (CH_3HgI). The granular deposits (HgS) were produced by the action of the electron beam. Note the dense accumulation of granules in the cortex (*co*) and correspondingly dense accumulations in the exocuticle layer (*exo*) of each cuticle cell. An especially dense layer (suggesting a high concentration of sulfur) is visible in each cell at *a*, and corresponds in its location with the layer present in FIGURE 20*c*. Note the layer of granules at the junction of the cuticle and the cortex.

its outer edge (*a*, FIGURE 20*c*), which suggests a high concentration of sulfur in this portion of each cell, a feature that can be correlated with the earlier suggestions of two layers in the exocuticle.[37,40,41] Further evidence that this layer exists was indicated by the examination of the cuticle of fibers in which all the disulfide bonds were reduced and then coupled with methyl mercuric iodide (CH_3HgI) yielding—$SHgCH_3$ groups. Under the action of the electron beam it was observed that a dense granulation rapidly appeared in the cuticle (and also in the cortex), presumably due to the decomposition of the —$SHgCH_3$ groups giving rise to resolvable particles of HgS.

In the cuticle cells the exocuticle layer is clearly outlined (FIGURE 20*e*). In addition, the outer edge is extremely dense (*a*, FIGURE 20*e*) forming a distinct layer about 0.1 μ thick and similar to that which stains with OsO_4. In view of the specific nature of the reaction between CH_3HgI and cysteine the distribution of the dense granules approximates the distribution of sulfur and it can be concluded that the cuticle contains sulfur, most of it being concentrated in the exocuticle, particularly in the outer layer. In contrast the endocuticle is nonkeratinous.

The densely staining outer edge of the exocuticle may become differentiated from the exocuticle during development or, alternatively, it may be deposited as a separate entity in the "empty" region that often is seen between the outer limit of the developing exocuticle and the cell boundary.[4,39]

The results of electron-staining of the cuticle layer using sodium plumbite[42] are similar to those obtained above with CH_3HgI and, together, they reinforce the conclusion that under certain conditions the deposition of OsO_4 portrays, fairly accurately, the distribution of sulfur.

Acknowledgments

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THE INNERVATION OF A HAIR FOLLICLE*

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Nerves that are destined to supply the hair follicles are arranged in a manner fundamentally similar to the dermal nerve networks. To the observer a network of dermal nerves appears to close down about the epithelial column of the hair follicle as it differentiates from the epidermis. The nerves of the hair follicle and the dermal nerve networks constitute almost all of the nerve tissue of the skin of mammals, and thus the significance of the endings in the hair follicle in sensation must be emphasized.

The basic plan of the innervation of the hair follicle has been recognized for years and may be observed in FIGURE 1. Classical studies by Arnstein,[1] Retzius,[2] Kadanoff,[3] and Tello[4] have provided complete and careful descriptions. Recently Weddell and Pallie[5] described the final terminations of the nerve about the hair follicle; for their observations they used the hyaluronidase-methylene blue method that they originated. Montagna and Ellis,[6] and Schmit and I[7] have described a cholinesterase reaction in the nerves of the hair follicle. I[8-10] have used silver, methylene blue, and cholinesterase techniques in studying the innervation of hair.

Myelinated nerve fibers that supply the hair follicles rise from deep in the dermis or subcutaneous tissue. In human skin the observed number of large fibers supplying the hair follicle varies from 5 to 12. These fibers approach from all quadrants and reach to that portion of the hair follicle below the junction of the sebaceous glands with the follicles. There the heavy myelinated nerve forms a network, and the rough varicose nerves course at random up and down about the follicle in the adjacent connective tissue. This network becomes continuous with a double nonmyelinated network situated higher and closer to the outer or external root sheath. An outer circular group of fibers and an inner longitudinal set of fibers constitute this double nonmyelinated network. The inner longitudinal fibers run both up and down the external root sheath and terminate freely. Occasionally some of these nerves rise to the neck of the hair follicles and enter into the formation of a network at this level. In heavy hair some of the final nonmyelinated nerves may have bulb-like expansions or netlike terminations. Miller and his associates[11] recently described this termination that appears to be an expression of the complexity of the nerve tissue rather than an indication of a specific function. It is possible for these endings to be invaginated between the cells of the external root sheath. This does not constitute a separate form of ending but rather a variation of the basic nerve ending at the external root sheath of the hair follicle (FIGURE 2*a* and *b*).

Bonnet[12] first demonstrated that the nerve supply to hair follicles varies directly with the size of the hair produced by the follicle. Large follicles may

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† The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

have as many as 30 parent fibers; small follicles may have only four or five. Vellus hair has a rudimentary structure supplying it, and the stiff hair of the dorsum of the fingers and chin is heavily innervated.

During the hair growth cycle, the nerve network remains intact. FIGURE 1 shows a telogen hair with a complete series of networks visible. The network is present throughout the entire cycle. However, it does expand and is larger about the larger follicle of anagen hair. During involution of the hair follicle

FIGURE 1. The double network of myelinated and nonmyelinated fibers about the neck of a hair follicle in the telogen phase of hair growth. Cat skin; stain in all figures except FIGURE 5*b* was frozen section silver. × 165.

following defluvium of the club hair, the relationship of nerve network to hair follicle is not disturbed. The empty hair follicles of hairless mice have the rudimentary, but complete, innervation of the filled hair follicle.

The early observers of the nerves of the hair follicle stated that the hair papilla was innervated. Bonnet[12] and later Weddell and his co-workers[13] observed that nerves were not present in the hair papilla. My observations confirm this viewpoint.

The Sensory Hair

Sensory hair is similar to the common hair in that it is a morphologic unit. It only differs in the degree of development of the individual components and

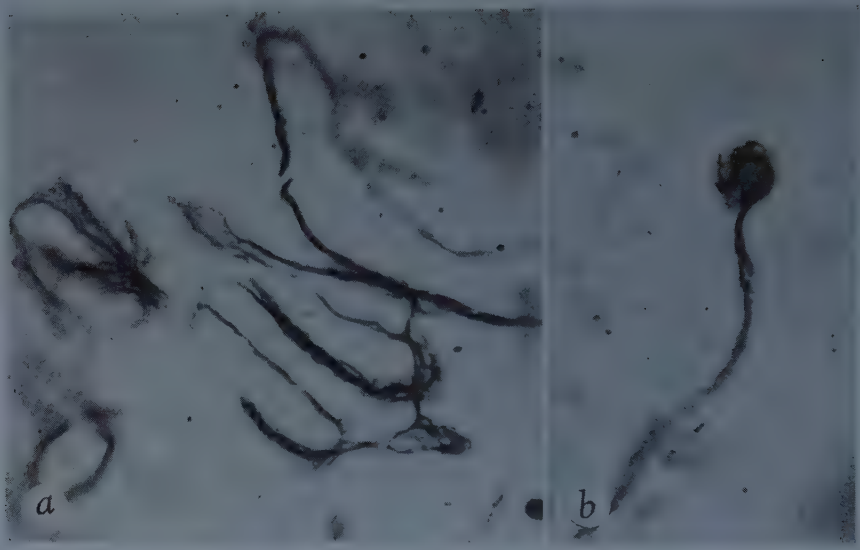

FIGURE 2. Netlike terminations in human skin: (*a*) about the external sheath of the hair follicle on the dorsum of the hand × 950; (*b*) about the hair follicle on the dorsum of the fingers. × 1000.

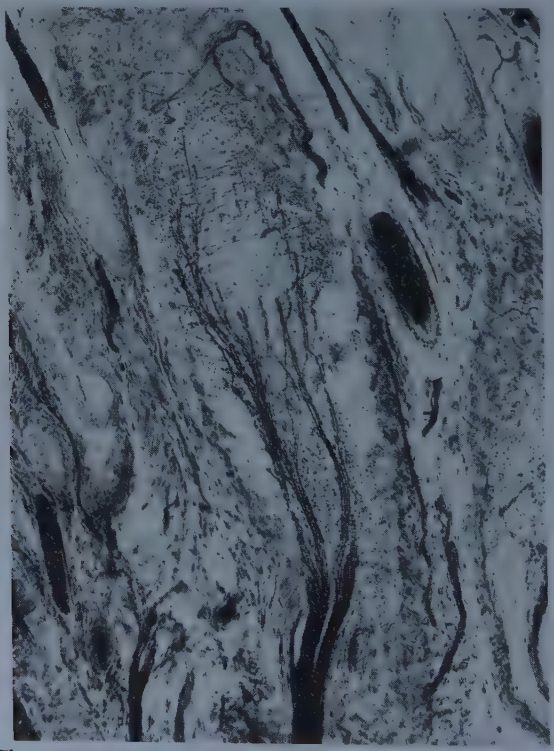

FIGURE 3. The nerves of the sensory hair of the mole's nose. Nonmyelinated and myelinated networks are visible. Direct contribution to the network at the neck of the hair follicle from the dermal nerve networks is visible. × 90.

in the presence of the connective tissue capsule that encloses the sinus. Colloquially, this type of hair is known as a whisker; it may be larger and longer than the usual hair. Such hairs are found in all the lower mammals and in the eyebrow of the gorilla, chimpanzee, and other primates studied to date. Man does not possess this structure.

Bonnet[12] originally recognized the morphologic difference between common hair and sensory hair. The large connective tissue capsule that envelops the

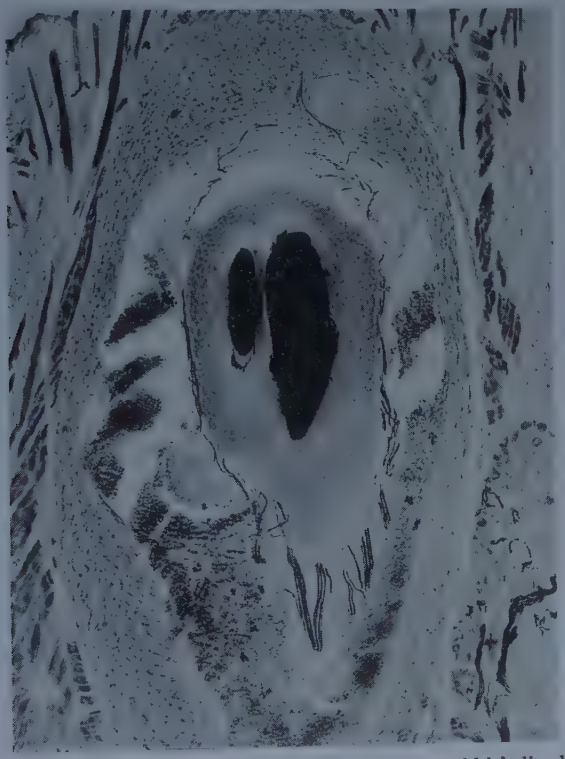

FIGURE 4. Nerve networks about the sensory hair of the rabbit's lip demonstrating the rough myelinated network in the cavernous sinus and the fine, nonmyelinated network at the neck of the hair follicles. × 60.

hair follicle and that contains the upper ring sinus and lower cavernous sinus is the distinguishing feature. Ostroumow[14] found leaflike nerve endings at the sensory hair. Botezat[15] also observed these endings and considered them similar to the tactile disks or Merkel's cells.

The sensory hair is innervated by two to five large nerve trunks that penetrate the connective tissue sac of the hair follicle at its base (FIGURE 3). These parent trunks separate into myelinated subdivisions that run through the vascular sinus to the external root sheath of the hair follicle. At the external root sheath a coarse myelinated network is formed (FIGURE 4). This courses toward the neck of the hair follicle, and at this point forms a circular nonmye-

linated network. Some of the nerves terminate in this area below the rudimentary sebaceous glands of the sensory hair follicles. Nerve networks are found outside the connective tissue capsule at the neck of the sensory hair, and this nerve penetrates the connective tissue capsule at the neck of the sensory hair follicle to join the fine circular nonmyelinated network. This can be observed at the top of FIGURE 3.

The final endings of the sensory hair appear to be fine nonmyelinated fibers. Merkel[16] stated that the endings were tactile disks. The disklike and platelike endings appear to me to be invaginations of the heavy myelinated nerves into the external root sheath. Miller and his associates,[11] however, recently stated that expanded nerve terminations consist of one type of nerve ending in the hair follicle, and the problems of network or bulb nerve endings are not settled.

The Tactile Disk

In 1905, Pinkus[17] described the tactile disk in association with the hair follicle. It is composed of a round, thickened epithelial plate, lying close to the hair follicle on the side toward which the hair shaft inclines. A myelinated nerve that ramifies immediately beneath it forms the nerve supply. The epidermal cells are thought to receive this innervation. Pinkus quoted F. Römer, who had observed tubercles in the epidermis of the echidna. Pinkus equated these eminences with the tactile disk and also found this structure in another monotreme, ornithorhynchus. He observed the structures in insectivora, rodentia, and primates. In man he found them all over the body, particularly on the lower part of the abdomen. In man they were 1 mm. or more in diameter. Kawamura[18] studied them in the abdominal skin of man with the aid of methylene blue and noted many different shapes. He suggested that these may be tactile endings. Earlier I suggested that such endings might be artifacts, but recently I have observed them in cat and other mammalian skin. The disk is readily demonstrated in the skin of hairless mice (FIGURE 5*a*). Straile[19] found it in rabbit skin. This ending gives a specific reaction to cholinesterase in the rabbit and guinea pig[10] (FIGURE 5*b*). I agree with Pinkus that it is analogous to the tactile spots of reptiles and amphibians.

Development

The dermal nerves are the first differentiated tissue of the developing skin. Nonmyelinated networks are organized formally in the dermis of the embryo before the epidermal appendages have begun to differentiate. I have observed this in the embryo of the rabbit. Tello[20] described similar findings in the skin of mice. To him the nerves seem to exert a distinct influence on the developing hair follicle, particularly the sensory hair. He thought that the maxillary branch of the fifth nerve had a direct influence on the formation of the whisker in the mouse. The influence of the embryonic nerve tissue on differentiation of other tissues is well documented by Hamburger.[21] This influence is apparently lost at later stages of growth.

In my work with R. Rosanove on rabbit embryos, we found that the nerve networks are well organized by the sixteenth day (FIGURE 6). By the twentieth day, the differentiated hair follicles are surrounded by the organized nerve

networks (FIGURE 7) and, by the twenty-second day, the networks of nerve fibers surrounding common and sensory hairs are complete in all details except the degree of myelination. No studies have been done as yet on the development of cholinesterase activity, nor has the development of the tactile disk been followed.

FIGURE 5*a*. Tactile disk of Pinkus from the abdominal skin of the hairless mouse × 440; (*b*) specific cholinesterase activity in the tactile disk of the rabbit's lip. × 150.

Function

The purpose of the hair, both common and sensory, is to increase the perception of the surface for tactile stimuli. The basic innervation appears to be myelinated A fibers. The close-set hair follicles, in furred mammals in particular, make this form of ending the primary sensory ending of the major portion of the skin. The innervation of the hair follicle is admirably suited to tactile sensation, for the hair shaft acts as a lever to increase the range of sensitivity following any minute mechanical change. The reaction of the tactile structures of the hair due to the presence of cholinesterase is not explicable. Such specific cholinesterase activity is present in the dermal nerve networks and in the tactile disks. It is not present in the Meissner corpuscle or other sensory end organs that give a nonspecific cholinesterase reaction. Yet in the

FIGURE 6. Nerve networks in the embryo of the rabbit of 16 days. Because of low magnification, the nerve near the epidermis is not readily visible. The nerve tissue is non-myelinated. × 90.

FIGURE 7. The nerve network about the developing hair follicle and within the connective tissue sheath of the sensory hair of the 22-day-embryo of the rabbit. × 300.

nerve networks of the hair follicles of the rabbit and the guinea pig, some nonspecific cholinesterase reaction is evident.[22] Hurley and Koelle[23] have shown that cholinesterase activity does not appear to be a factor in sensory perception. It may be a vestigial activity of the enzymes, or it may indicate general esterase reactions of nerve tissue.

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NEWER FINDINGS ON THE ENZYMES AND PROTEINS OF HAIR FOLLICLES

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There would appear to be three general methods of approach for the biochemist to use in studying the enzymes, proteins, and other components of the variety of cells that constitute hair follicles.

There is the cytochemical approach[1,2] that can provide valuable qualitative information about the occurrence and localization of many tissue constituents. Quantitative studies are more difficult however, and the number of specific reagents available is limited. Nevertheless, this approach has the advantage that all the layers of the follicle can be studied.

Another technique that could be employed is the application of quantitative histochemical methods, such as those of the Linderström-Lang school[3] to single follicles, microdissected portions of follicles and skin slices.

Finally, large quantities of follicle material may be prepared from the flayed skin of the sheep by the wax sheet method.[4] Material prepared by this method is referred to as "wool roots," each root consisting of most of the bulb, the prekeratinized portions of the fiber and, frequently, the attached inner root sheath (FIGURE 1). Wool roots can be investigated[5] by the many refined biochemical methods devised for the study of other animal tissues. However, the results of such an approach apply only to cells of the developing fiber itself and, possibly, to those of the inner root sheath, but not to those of the outer root sheath.

Some experiments performed with extracts of wool roots prepared by the wax sheet technique are described in this report.

Biochemical Studies of the Wool Root

Preparation of Wool Roots

As soon as possible after slaughter, sheepskins are transported to the laboratory where the wool is shorn with fine animal clippers close to the skin. The skin is then refrigerated at +1° C. for 30 min. A thin layer (0.5 to 1 cm. thick) of the wax mixture of Ellis[4] at a temperature not greater than 55° C. is poured onto the shorn surface of the skin and allowed to stand for 10 min. to solidify. By carefully pulling the skin away from the wax sheet, the wool roots are plucked from the skin and are held in the wax by their shafts. They can be harvested with fine clippers and immediately stored in a water-saturated atmosphere at +5° C. Approximately 10,000 sq. cm. of sheepskin can be conveniently treated in this manner in each experiment with an average yield of wet wool roots of about 2 mg./sq. cm. The typical appearance of wool roots obtained by this method is shown in FIGURE 1.

Free Amino Acids in Aqueous Extracts of Wool Roots

Solutions essentially free of proteins but containing free amino acids and related compounds were prepared by homogenizing wool roots with water in a

Waring Blendor (FIGURE 2) and adding sufficient ethanol to the homogenate to bring the concentration to 70 per cent. The extract was centrifuged, evaporated to dryness in a rotary film-evaporator, reconstituted to a small volume and centrifuged again. This extract was suitable for chromatographic analysis. Similar extracts were also prepared by picric acid extraction followed by removal of the picric acid on Dowex-2 resin.[6]

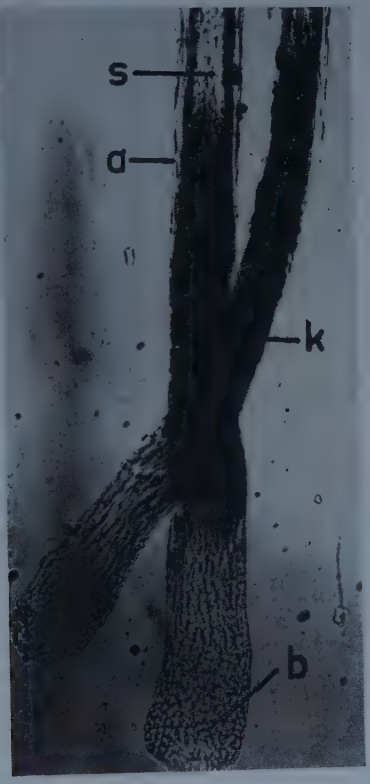

FIGURE 1. Wool roots plucked from sheepskin by the wax-sheet method. Attached inner root sheath at *a*, otherwise the wool root consists of bulb *b*, keratogenous zone *k* and keratinized shaft *s*.

A typical two-dimensional chromatogram of a wool-root extract (200 μg. N/ml.) is shown in FIGURE 3 and the results of a quantitative analysis by the Moore and Stein ion-exchange technique[7] are given in TABLE 1. Some of the Ninhydrin-positive spots on the paper chromatograms could not be resolved and identified in the quantitative analysis.

More amino acids and other Ninhydrin-positive substances were detected in the present investigation than in a previous study.[5] All the amino acids of hair and wool keratin were present. Of special note are the high content of glutamic acid (TABLE 1) and the presence of glutamine and asparagine, all of which are prominent free constituents of cells generally. Proline is present in significant quantities (TABLE 1) as also are glycine, serine, and the basic amino

FIGURE 2. Homogenate of wool roots in water. The soft portions of the roots have disintegrated giving a suspension of whole and disrupted cortical cells and keratinized fiber shafts (*s*) with "brush" ends.

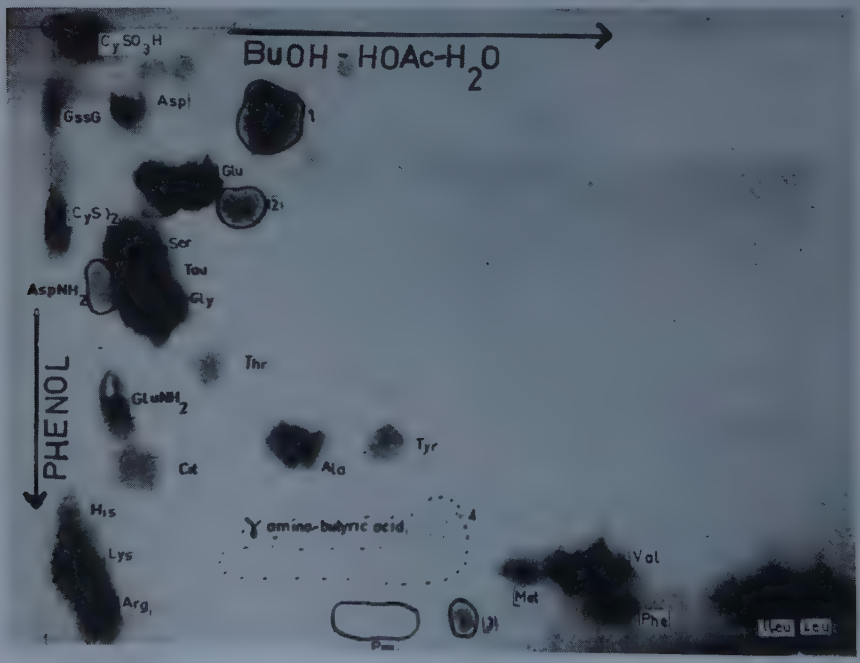

FIGURE 3. Two-dimensional chromatogram of free amino acids and other Ninhydrin-positive compounds in aqueous extracts of wool roots. Solvents: *n*-butanol/acetic acid/water-4:1:1, and 75 per cent aqueous phenol.

TABLE 1

QUANTITATIVE ANALYSIS OF FREE AMINO ACIDS IN WOOL ROOTS

Amino acid	Content μg. amino acid per gm. wool roots‡	Amino acid	Content μg. amino acid per gm. wool roots‡
Alanine	42.9	Proline	50.0
Ammonia	(38.4)	Serine	
Arginine	93.3	Threonine *	(26.2)
Aspartic acid	25.7	Asparagine	
Citrulline	17.3	Glutamine	
Cysteic acid	38.0	Tyrosine	37.7
Cystine *	(8.1)	Valine	49.8
Glutathione		Unknown 1	(5.74)
Glutamic acid	296	Unknown 2 †	(2.11)
Glycine	144	Unknown 3 †	(6.19)
Histidine	38.4	Unknown 4 †	(3.48)
iso-Leucine	36.9	Unknown 5, (probably methionine)	22.8
Leucine	63.2		
Lysine	76.2	Unknown 6 (probably γ-amino butyric acid)	8.3
Phenylalanine	37.7		

* All are present (FIGURE 3) but were not separately resolved.

† Eluted after cysteic acid. Unknown 4 probably is taurine and unknowns 1 and 3 may correspond with spots 1 and 2 in FIGURE 3.

‡ The analytical figures quoted in parentheses are those that can be expressed only as μg. N per gm. wool roots (the same color yield as that of leucine was assumed).

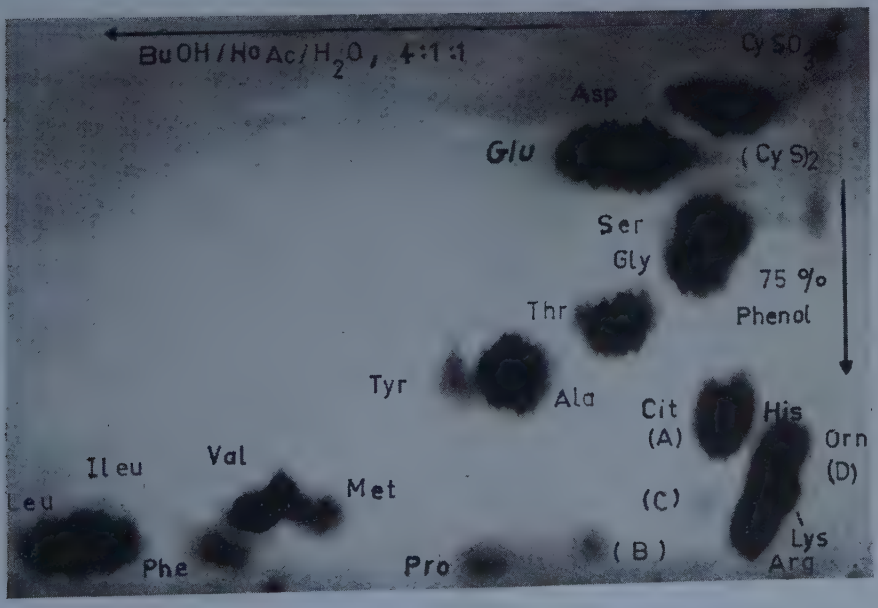

FIGURE 4. Two-dimensional chromatogram of an acid hydrolyzate of inner root sheaths from vibrissal follicles of the rat.

acids. Free citrulline was positively identified by its color reaction with Ehrlich's reagent and the position of its peak in the quantitative analysis. It was not possible to determine by paper chromatography whether glucosamine is present, since it migrates to the same position as citrulline.

Cysteic acid, cystine, and methionine are present but only in very small amounts (TABLE 1). The presence of glutathione (FIGURE 3) was established by comparison with control paper chromatograms on which authentic samples had been run. At least four Ninhydrin-positive spots have not been identified. Spots 1 and 2 (FIGURE 3) are in unusual positions and, although they are acidic as they were removed, together with cysteic acid, glutathione, and glutamic and aspartic acids, by treatment of the extracts with Dowex-2, they have not yet been identified. Spot 4 is in the region of the paper where β- or γ-amino butyric acids are to be found. There are no clues as to the identity of spot 3, near proline. It is not the same as spot B that occurs in hydrolyzates of the inner root sheath (FIGURE 4).

The results show that wool roots contain a pool of amino acids that presumably are available for the synthesis of keratin. The lack of peptides, with the exception of small amounts of glutathione, is perhaps surprising although small peptides generally are not abundant in extracts of animal tissues. If they are intermediates in protein synthesis, peptides could be expected to have a very short life. Similarly, cystine itself is present in only small amounts (TABLE 1) and was not found previously.[5] Probably there is a high demand for cystine by the growing fiber and the mechanisms of the cell are geared to maximum utilization of sulfur-containing amino acids.

Enzymes in the Wool Root: Transamination and Transpeptidation

A number of enzymes have been identified[5] and localized histochemically in hair follicles.[1,2,5,8] However, apart from autoradiographic studies with labeled-amino acids[9,10] and some histochemical results,[2,5] relatively little is known about the metabolism of amino acids and proteins in the follicle despite the fact that this tissue is primarily concerned with the synthesis of proteins.

Ellis *et al.*[5] were led to suggest that enzymes concerned with transamination might be present in wool roots and, more recently,[11] it has been established that both transamination and transpeptidation reactions indeed do occur in homogenates of this tissue.

For transamination, buffered (*p*H 7.4) homogenates of wool roots partially clarified by filtration and centrifugation were incubated with appropriate α-oxo acids and α-amino acids in the presence of added coenzyme, pyridoxal phosphate. After incubation the reaction was stopped by addition of ethanol and the reaction mixtures finally chromatographed on paper with 80 per cent phenol. Thus far the formation of alanine from pyruvic and aspartic acids, and of glutamic acid from α-oxoglutaric acid and alanine or citrulline has been demonstrated. It is of interest that citrulline, which occurs in the free state in wool roots as well as combined in the inner root sheath[12,13] participates in transamination.

Evidence for the occurrence of transpeptidation (γ-glutamyl transferase[14,15]) in wool roots was obtained by chromatography. Thus the formation of γ-glutamyl glycine was detected following the incubation of wool-root homog-

enates with glutathione and glycine. In addition to transpeptidase, paper chromatograms indicated that wool roots probably also possess protease and peptidase activity; however, cysteinyl-glycinase activity, which frequently accompanies transpeptidation, was weak or absent. It is of interest that Haurowitz and his co-workers[16] have recently reported evidence for the occurrence of γ-glutamyl residues in wool. The transpeptidase mentioned above could conceivably play a part in the formation of such structures.

The two types of enzymatic activity concerned with amino acid metabolism, transamination and transpeptidation, are additional to those enzyme reactions previously detected in hair follicles. These enzyme activities when considered together with the numerous free amino acids, which have been found in wool roots, especially glutamic acid, glutamine, glycine, and alanine, suggest that the cells of the hair root are capable of synthesizing various amino acids necessary for the synthesis of keratin.

Isolation of a Fibrous Precursor of Keratin from the Wool Root

Extraction with urea. Strong solutions (8 M) of urea rapidly swell and disrupt the cells of the bulb and keratogenous zone of the wool root yielding a protein solution that gives an intense reaction with alkaline nitroprusside and is thus rich in —SH groups. The —SH content* is about 500 μmoles per gram of dissolved protein. The residual wool roots contain very few —SH groups and consist of keratinized shafts to which inner root sheaths are frequently found to be attached (FIGURE 5). Except for the trichohyalin granules the protein in the cells of the inner root sheath are not dispersed by 8 M urea.[12] From histological and other experiments carried out it was clear that the protein dispersed by 8 M urea was derived chiefly from the fibrillation zone of the wool root. Thus, under the light microscope, birefringent fibrils could be seen to disperse rapidly in the presence of urea; moreover, although the cells of the bulb region were disrupted and their contents dispersed it was found that the soluble proteins of this region were denatured by treatment with urea and became insoluble on its subsequent removal by dialysis.

When preparing quantities of wool-root protein by dispersion with urea it was observed that the —SH reactive protein precipitated as a fibrous gel on dialysis against water. At the same time the —SH content diminished and the precipitated protein could be redissolved only in 8 M urea at alkaline *p*H values. If the protein was freeze-dried, the addition of thioglycollic acid to the alkali became necessary in order to render it soluble again. These changes were similar to those observed with proteins extracted from wool by alkaline thioglycollate solutions that have been attributed to protein aggregation resulting from the auto-oxidation of —SH groups.[17]

Also, as for the wool proteins, it was found that if the —SH proteins extracted from the wool root in the presence of 8 M urea were coupled immediately with iodoacetic acid at *p*H 8, either during or immediately after extraction, then the majority of the —SH groups could be converted to S-carboxymethyl groups (—S—CH_2—COOH):

$$\text{protein}\text{—SH} + \text{I—}CH_2\text{—COOH} \rightarrow \text{protein}\text{—S—}CH_2\text{—COOH} + \text{HI}$$

* Amperometric titration by S. J. Leach.[16a]

The resultant proteins now contained S-carboxymethylcysteine residues instead of cysteine residues, and they remained water-soluble even after dialysis and freeze-drying. They were amenable to study by physicochemical methods since the only chemical change was the replacement of each —SH group by a more readily ionizable —S—CH_2—COOH group.

The proteins in the solutions, when freed of urea by exhaustive dialysis against water, were found to precipitate at two *p*H values, the bulk coming out of solution as a highly-hydrated gel at *p*H 5.2, and the remainder at *p*H

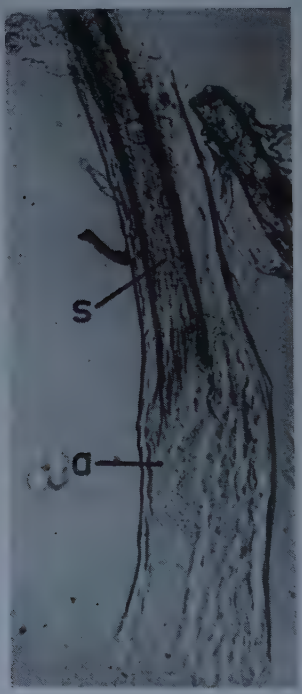

FIGURE 5. Wool root after treatment with 8 M urea for 30 min. The bulb region and keratogenous zone have dispersed leaving keratinized shaft *s* and inner root sheath *a*.

3.5. This initial *p*H fractionation was utilized in the routine method for preparing wool root-proteins according to the scheme outlined in FIGURE 6.

Details of three preparations (P3, P4, and P6) are given in TABLE 2. From examination of a number of such preparations it was determined that the average total yield of freeze-dried protein from wet wool roots approximates 14 per cent, and that 60 to 70 per cent of the protein was precipitated at *p*H 5.2. This protein fraction (Fb) had a surprisingly low total-sulfur content compared with that of the protein fraction (Fa) which precipitated at *p*H 3.5 (TABLE 2), and compared with the total-sulfur content of wool itself (approximately 3.5 per cent).

It may also be seen from TABLE 2 that the cystine contents of the two protein fractions are extremely low, indicating that more than 90 per cent of the

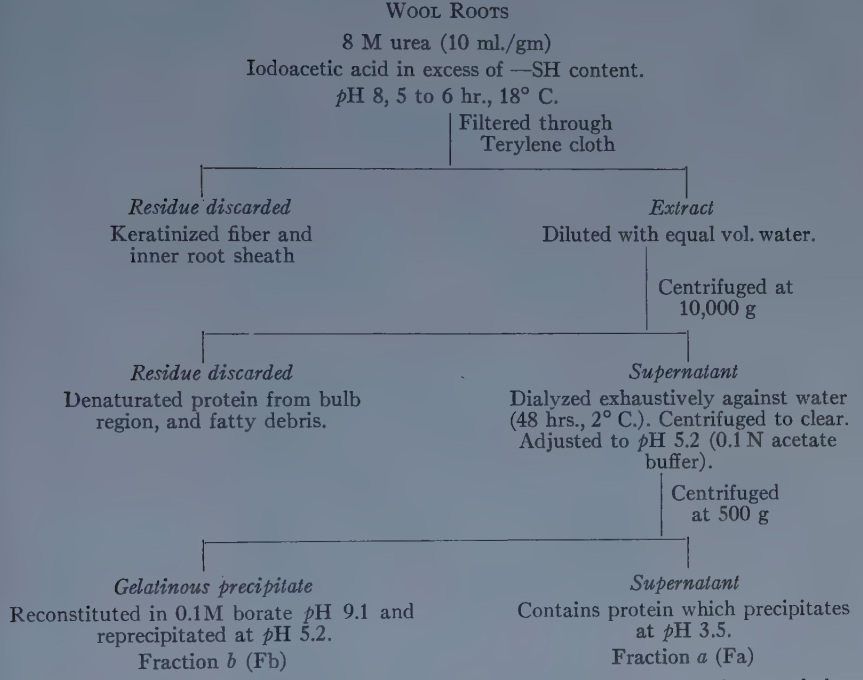

Figure 6. Summary of fractionation procedure for preparing S-carboxymethyl proteins from wool roots.

Table 2

Proteins (S-Carboxymethyl Derivatives) Isolated from Wool Roots by Extraction with 8 M Urea

Preparation	Yield of protein§ (percentages)	Description of fractions	Protein in fractions (percentage totals)	Total S* (percentages)	Cystine content† (percentages)
P3Fb	14.2	pH 5.2 ppte	72	‡	‡
P3Fa		pH 3.5 ppte	28	‡	‡
P4Fb	14.8	pH 5.2 ppte	65	1.60	0.2
P4Fa		pH 3.5 ppte	35	4.00	1.2
P6Fb	14.2	pH 5.2 ppte	62	1.79	0.1
P6Fa		pH 3.5 ppte	38	4.13	0.6

* Analysis by R. W. Zimmerman, Microanalytical Service, Melbourne, Australia.
† Amperometric titration performed by S. J. Leach.
‡ Not analyzed.
§ Freeze-dried protein in dialyzed urea extract (total protein) per cent of wet weight of wool roots.

sulfur reacted with iodoacetic acid giving S-carboxymethylcysteine residues. On hydrolysis the proteins yield the free amino acid S-carboxymethylcysteine which has been identified in the Moore and Stein ion-exchange analysis of amino acids (TABLE 4).

Moving boundary electrophoretic analysis of the protein fractions was informative (FIGURE 7*a–d*, TABLE 3). Electrophoresis in glycine—NaOH buffer,

TABLE 3

MOVING-BOUNDARY ELECTROPHORETIC ANALYSES OF S-CARBOXYMETHYL PROTEINS ISOLATED FROM WOOL ROOTS WITH 8 M UREA

Glycine-NaOH buffer, *p*H 10.9 (I = 0.1); Protein Concentration 1 Per Cent

Preparation	Observed mobilities (u) and distribution of protein (c) in electrophoresis diagrams									
	Peak A		Peak B		Peak C		Peak D		Peak E	
	$u \times 10^5$	c %	$u \times 10^5$	c %	$u \times 10^5$	c %	$u \times 10^5$	c %	$u \times 10^5$	c %
P3 Urea extract	*		5.4	29	6.6	58	8.6	11	~13	2.5
P3Fb *p*H 5.2 ppte	*		5.4	31	6.6	64	—	—	~13	2.3
P3Fa *p*H 3.5 ppte	4.8	12	—	—	7.1	28	8.2	52	~13	8
P4Fb *p*H 5.2 ppte	*		5.5	22	6.6	73	—	—	—	—
P6Fb *p*H 5.2 ppte	*		5.4	23	6.0	77	—	—	—	—

* The slow moving peak A was present in very small amounts.

TABLE 4

COMPARISON OF THE AMINO ACID COMPOSITION* OF WOOL AND THE LOW-SULFUR PROTEIN† OF WOOL ROOTS

Amino acid	Low-sulfur protein	Whole wool‡
Alanine	4.76	4.04
Amide-N	7.74	6.57
Arginine	19.48	18.32
Aspartic acid	6.54	4.64
Cystine	3.18§	6.99
Glutamic acid	9.26	7.18
Glycine	5.30	5.58
Histidine	2.37	1.41
iso-Leucine	2.89	1.62
Leucine	7.14	4.69
Lysine	7.00	3.60
Methionine	—	0.31
Phenylalanine	2.00	1.76
Proline	2.71	5.61
Serine	5.34	7.39
Threonine	3.40	4.06
Tyrosine	2.37	2.54
Valine	3.77	2.57

* Amino acid nitrogen as a percentage of total nitrogen.
† S-carboxymethyl derivative.
‡ Sample typical of sheepskins from which wool roots were obtained.
§ Cystine equivalent to S-carboxymethylcysteine.

*p*H 11, ionic strength 0.1, of the total urea-extractable proteins before *p*H fractionation showed the presence of at least five components (FIGURE 7*a*), the major peak (C) accounting for about 60 per cent of the total area of the

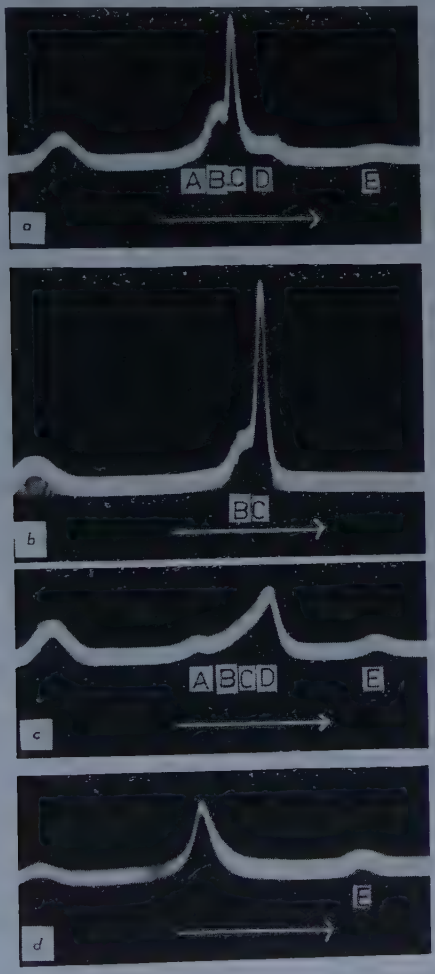

FIGURE 7*a–d*. Ascending electrophoretic patterns of wool root protein fractions run in glycine-NaOH buffer at *p*H 11, ionic strength 0.1; (*a*) total urea-extracted proteins; (*b*) urea extract, protein precipitated at *p*H 5.2 (Fb); (*c*) urea extract, protein not precipitated at *p*H 5.2 but precipitated at *p*H 3.5 (Fa); and (*d*) proteins extracted with water. No urea.

diagram. Generally, it was found that the fractionation at *p*H 5.2 removed the faster moving components, peaks D and E, and partially reduced the amount of the slower moving material, peaks A and B (FIGURE 7*b*), these components appearing in the proteins of the supernatant, which precipitate at *p*H 3.5 (FIGURE 7*c*). The greater mobility of peaks D and E can be correlated with the greater charge on the proteins. This would be conferred by a large

number of S-carboxymethyl groups equivalent to the high sulfur content of this fraction (Fa). The need to adjust the *p*H value to a lower value for precipitation would likewise be explained by the many carboxyl groups present.

In TABLE 3, the peak C component in preparation P3Fa is seen to have a higher mobility than peak C in the other preparations but they probably are identical. In the proteins that precipitate at *p*H 5.2 and have a low sulfur content the major peak C represents 70 to 80 per cent of the total area. It is clearly defined on its leading edge (FIGURE 7*b*) but it is associated with about 20 per cent of the slow-moving component B. This type of electrophoresis pattern was consistent in preparations of the low-sulfur protein from different batches of wool roots (P4Fb, P6Fb, TABLE 3) and further fractionation would be expected to yield an electrophoretically-homogeneous preparation of the major component.

It is interesting to note that the presence of two protein fractions having low and high sulfur contents and different precipitation points closely resembles the picture for the proteins extracted from epidermis[18] and from wool under certain conditions.[19-21] In the present case it is reasonable to conclude that the low-sulfur protein (Fb), or perhaps the protein of peak C itself, is derived from the microfibrils of the fiber cortex. Thus the protein not only has conspicuous fibrous properties but it originates almost entirely from cells of the fibrillation zone of the wool that have been well-characterized by electron microscopy (see Rogers[57]). These studies have shown that the principal cytoplasmic component consists of macrofibrils of keratin, which themselves are made up of microfibrils embedded in an amorphous sulfur-rich matrix. The nonfibrous γ-keratose obtained from oxidized wool contains 4 to 6 per cent of sulfur; it also derives from this matrix.[19,22] The high sulfur protein (Fa), or possibly the protein of electrophoresis peaks D and E obtained from wool roots, probably has the same origin.

The low cystine content of the S-carboxymethylated proteins of the wool root (TABLE 2), especially in the low-sulfur fraction, indicates that the proteins as they occur in the keratogenous zone of the root, are almost completely (>90 per cent) in the reduced form and that disulfide bonds are almost absent. The small amount of cystine found to be present could have arisen by auto-oxidation during isolation of the wool roots and the proteins. However, the slightly higher figure for cystine in the high-sulfur fraction suggests the probability that some disulfide bonds are present in this particular protein, in the wool root. These results do not accord with the histochemical data,[1,2] which indicate a high concentration of disulfide bonds in the keratogenous zone. This discrepancy points to a lack of specificity of the histochemical method.

Examination of the low-sulfur protein in the ultracentrifuge* revealed the presence of a quantity of heavy material that settled out rapidly. The remainder of the protein sedimented as a fairly distinct peak (FIGURE 8) although small quantities of lighter and heavier material were still associated with it. At protein concentrations of 0.65 per cent and 1.3 per cent, *p*H 6.6 (phosphate-NaCl) and ionic strength 0.19, the sedimentation coefficients were 4.07×10^{-13} and 3.35×10^{-13} respectively.

* Spinco, Model E.

Very little can be deduced from the sedimentation patterns regarding the molecular size of the protein. The protein showed varying degrees of aggregation, and this polydispersity could be reduced by hydrogen-bond-breaking agents such as urea. Keeping in mind this tendency of the protein to aggregate and also the present lack of other data, it can be said that the S_{25w} values of the main peak are consistent with a molecular weight of about 100,000.

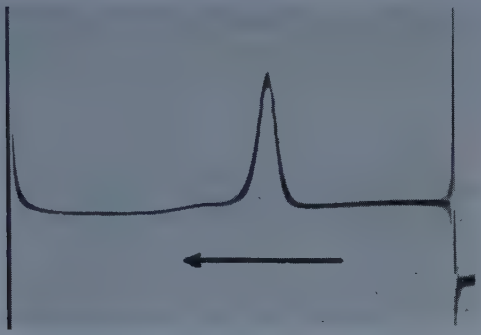

FIGURE 8. Sedimentation pattern of low-sulfur protein (Fb) from wool roots run in phosphate-NaCl buffer at *p*H 6.6, ionic strength 0.19, protein concentration 0.65 per cent. Time 100 min.; speed 56,000 rpm; angle 65°.

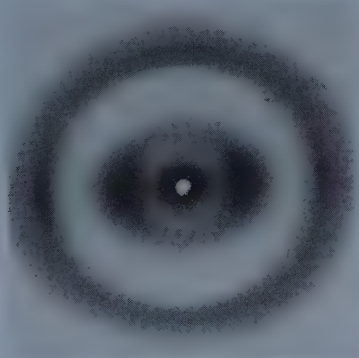

FIGURE 9. X-ray diagram of stretched film of low-sulfur protein from wool roots. A highly oriented α-pattern predominates but parallel-β, cross-β reflections and some low-angle scatter near the center of the diagram are also present.

That the low-sulfur protein consists of fibrous molecules was indicated by other physical properties. Thus films of the protein prepared on nonpolar glass could be stretched approximately 200 per cent in saturated salt solutions. They were washed in 70 per cent ethanol and absolute ethanol and dried in air. The films were highly oriented as evidenced by their birefringence and by the highly-oriented X-ray patterns that were largely of the α-type (FIGURE 9) although traces of β and cross-β reflections were also present. Indications of low-angle X-ray scatter in the equatorial region suggest the presence of fibrous aggregates larger than polypeptide chains.

Solutions of the protein have not been studied by electron microscopy but a preparation of the gel precipitated at *p*H 5.2 was examined and it is of interest

to compare its globular appearance (FIGURE 10) with the electron micrographs of Farrant *et al.*[23] of globular particles prepared from enzymatic digests of wool.

Although the low-sulfur protein was not electrophoretically pure, it was of interest at this stage to compare its quantitative amino acid composition with that of wool. From TABLE 4 it may be seen that the content of SCM-cysteine is low, a fact already established from the low sulfur content. Relative to whole wool the protein contains smaller amounts of the amino acids cystine, serine, threonine, and proline (so-called polar amino acids) but a high content of the acidic, basic and neutral amino acids. Similar general trends in amino acid composition have been observed for keratin derivatives extracted from wool.[21,24,25]

FIGURE 10. Electron micrograph of gel of low-sulfur protein (Fb) from wool roots, formed at *p*H 5.2. Shadowed with uranium. Globular particles 100 to 200 Å dia. can be seen.

Since the fibrous protein almost certainly originates from the microfibrils it follows that the concentration of amino acids with polar side chains is low in these structures, whereas the content of acidic, basic and neutral amino acids is high. Consequently the content of cystine, serine, threonine, and proline must be higher in the amorphous matrix and therefore in the proteins derived from this region. Analyses reveal that this is true for γ-keratose[25,26] and if, as suggested, the matrix is the source of the high-sulfur protein from wool roots, then a similar trend should be found for this protein also. Analyses to check this possibility have yet to be done.

The trends in the distribution of amino acids between the microfibrils and the matrix discussed above do not accord with the recent suggestion of Ghosh *et al.*[27] that the polar, basic, and acidic amino acids are absent from the crystalline regions and present only in the amorphous phase.

Extraction with lithium bromide. The swelling and dispersion of wool roots in 75 per cent lithium bromide was slower than in 8 M urea, so that after the addition of iodoacetate it was necessary to disperse them in a Waring Blendor

in order to obtain solutions of wool-root proteins. The solutions obtained were more viscous than the corresponding urea solutions. Electrophoretic analysis revealed peaks similar to those obtained with urea although the mobilities were slightly lower.

Extraction with water. Treatment of wool roots with water in a Waring Blendor followed by high-speed centrifugation yielded a solution that gave a weak reaction for —SH groups. Iodoacetate at *p*H 8 was added and allowed to react until the nitroprusside test was negative. The main peak of the electrophoresis pattern was complex (FIGURE 7*d*) but the fast peak ($u \sim 13$) was probably identical with peak E. The proteins extracted by water are probably globular proteins largely derived from the bulb region and they could contain

FIGURE 11. Electron micrograph of microfibrils isolated from wool root homogenates by differential centrifugation. Shadowed with uranium.

globular-type keratin precursors. However, this need not be true, for the mechanisms of synthesis and fibrogenesis may be such that the primary particle has only a short life in the cell. From the same aqueous homogenates of wool roots (FIGURE 2), microfibrils were isolated by differential centrifugation (FIGURE 11); these will be studied by physicochemical methods and with the electron microscope.

The Structure and Composition of the Inner Root Sheath

From histochemical studies of the inner root sheath[1,2,28] it has been assumed that protein is the principal constituent of both the trichohyalin granules and the birefringent component. Under the electron microscope the fibrous component of the cells of the inner root sheath is seen to consist of fine filaments (~60Å dia.: see FIGURE 12) that resemble the microfibrils of the hair cortex. Although a proportion of these filaments apparently derive directly from the amorphous trichohyalin granules[29] it is also likely that the final rapid filling of

the cytoplasm with filaments, which gives rise to the hyalinized cells, takes place without the intermediate formation of trichohyalin granules.[30]

An investigation was undertaken to compare the composition of these cells in the three layers of the sheath with those of hair.[12,13] Vibrissal follicles plucked from the snout of the rat were found to constitute a convenient source of the sheaths. It was also found that the distal portion of the sheath or the whole sheath could be dissected from about 20 per cent of the plucked vibrissae under a dissecting microscope. The vibrissae were dissected into 0.9 per cent

FIGURE 12. Electron micrograph of a thin section of the inner root sheath from a rat vibrissal follicle. Microfibrils of fibrous protein oriented in portion of a cell can be seen. OsO_4 fixation.

NaCl, (FIGURE 13) but those used for analysis were also routinely washed with 8 M urea, with water, and finally dried. In this way about 2500 follicles were dissected and 15 mg. of dried sheath material was obtained. The tedious nature of the process precluded any attempt to dissolve the birefringent component out of the cells.

The dissected sheaths were excellent subjects for light microscopy and a number of properties of the trichohyalin granules and the fibrous component were investigated.[12] Urea (8 M) dissolved the granules but the hyalin cells were swollen only slightly with a lowering of the birefringence, and this was restored by washing with water. A similar swelling effect has been observed with 98 to 100 per cent formic acid. Of particular interest was the action of proteolytic enzymes that caused a rapid separation of the cells and digestion

of their birefringent contents leaving membranous "ghosts" derived from the undigested cell membranes (FIGURE 14).

The fibrous component is the major constituent of the cells of the inner root sheath. Two-dimensional paper chromatograms of 9-hour hydrolyzates (6 *N* HCl, 105° C.) of the dissected sheaths revealed the presence of an array of

FIGURE 13. A group of birefringent inner root sheaths dissected from vibrissal follicles of the rat, treated with 8 M urea and washed in water. Polarized light.

FIGURE 14. Inner root sheath cells after the action of pepsin (crystalline, 5 mg./ml.), 10 min., *p*H 2, 40° C. The cells have separated from one another, and most of their birefringent contents have been digested. Only the cell membranes and nuclear remnants remain. Phase-contrast.

amino acids, thereby confirming the sheath's protein nature. X-ray diffraction patterns showed that the fibrous protein is of the α-type but the absence of discrete reflections at low angles indicated that, unlike the microfibrils of hair, the packing of the filaments of the protein is poorly crystalline. Rudall[31] has reported that the inner root sheath of the porcupine-quill follicle gives an α-type X-ray diagram.

All the amino acids present in hair are also present in the inner root sheath but in different proportions (FIGURE 4). Of special note was the extremely small amount of cystine present (cystine + cysteic acid), the presence of methionine, and the intense glutamic acid spot. In addition, 4 Ninhydrin-positive spots (A, B, C, and D) not found in hydrolyzates of hair were observed. The amino acids of spots A and D that run with the basic amino acids were identified as citrulline and ornithine respectively, and C is probably methionine S-oxide, since methionine itself is present. Spot B remains unidentified. The citrulline in Spot A was identified by extensive tests that included the produc-

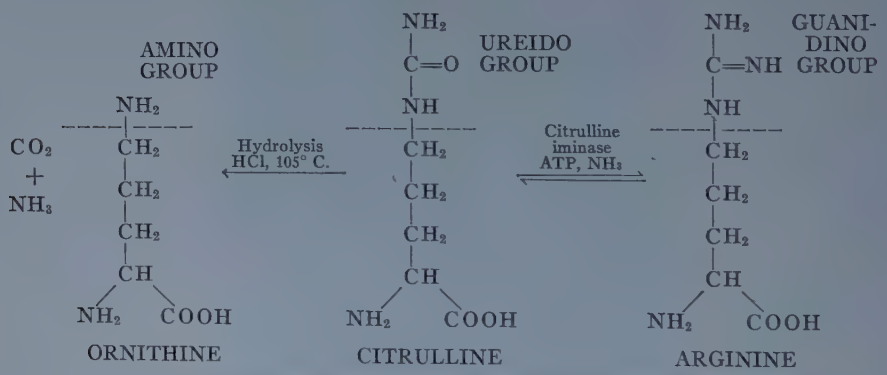

FIGURE 15. Formulas and some reactions of arginine, citrulline and ornithine.

tion of a characteristic yellow color with Ehrlich's reagent, the spot being Ninhydrin-positive after this treatment, and comparison of the R_f value of the eluted spot re-run in a variety of solvent systems with authentic L-citrulline. Glucosamine, which does not separate from citrulline in the two-dimensional chromatogram was eliminated as a possible component by the absence of a color reaction specific for this substance. The presence of citrulline was also indicated by the intense red coloration produced in inner root sheaths when they were treated with the diacetylmonoxime reagent of Fearon[32] and by the intense yellow color produced when Ehrlich's reagent (in 1 *N* HCl) was applied. The amino acid of spot D was identified as ornithine by comparison with the spots obtained in control experiments in which citrulline was hydrolyzed with 6 *N* HCl at 105° C. (FIGURE 15). The intensity of the ornithine spot on the paper chromatograms was found to decrease as the time of hydrolysis was decreased. No proline was formed, contrary to the claims of Wada.[33] The fact that ornithine is not present in the inner root sheath but occurs in hydrolyzates only as a product of the hydrolysis of the citrulline present was indicated by the absence of δ-DNP-ornithine in hydrolyzates of the inner root sheath treated with 1-fluoro-2,4-dinitrobenzene (FDNB).

A quantitative analysis of the amino acids in hydrolyzates of the inner root sheath was carried out[13] by the Moore and Stein ion-exchange technique.[7] The results are given in TABLE 5, and comparison can be made with the analyses of the associated vibrissal hairs and their roots.

The peak in the analysis attributed to citrulline was confirmed by running authentic L-citrulline. Citrulline is one of the most abundant amino acids in the sheath. As indicated by the paper-chromatograms, the content of glutamic acid also is high and, together with aspartic acid, it makes up about 26 per cent

TABLE 5

AMINO-ACID COMPOSITION OF HYDROLYZATES OF HAIRS, HAIR ROOTS, AND INNER ROOT SHEATHS FROM VIBRISSA FOLLICLES OF THE RAT

Per cent by Weight of Sample Dried at 60° C. Over Phosphorus Pentoxide

Amino-acid	Hair	Hair root	Inner root sheath
Alanine	3.5	3.2	3.1
Amide	1.2	1.2	0.4
Arginine	7.8	7.7	5.1
Aspartic acid	5.8	6.1	7.1
Cystine	13.7	7.4	1.0
Glutamic acid	11.6	11.4	>18.8*
Glycine	5.1	4.2	2.9
Histidine	1.3	1.4	1.0
iso-Leucine	1.9	2.0	1.6
Leucine	5.5	6.0	7.1
Lysine	3.1	3.2	2.8†
Methionine	—	—	0.1
Phenylalanine	2.7	3.2	2.7
Serine	8.6	6.7	3.9
Threonine	4.6	3.9	2.4
Tyrosine	4.4	4.1	2.1
Valine	3.1	2.9	1.8
Citrulline	—	—	6.1
	83.9‡	74.6‡	70.0‡

* This must be regarded as a minimum figure since the optical density exceeded the limit for accurate reading by the equipment.

† Including ornithine produced from citrulline breakdown.

‡ The recoveries are all low. The analyses do not include tryptophan and proline, and have not been corrected for material not containing protein, such as pigment, nucleic acids, lipids and carbohydrates that are present to an unknown extent.

of the protein. Cystine is present in only small amount, but methionine is present to a larger extent than in hair. In general the protein appears to be rich in polar side chains. Its stability however, in contrast to keratin, is apparently not attributable to disulfide linkages but to the influence of secondary forces such as salt links and hydrogen bonds.

The presence of citrulline has been demonstrated in the inner root sheaths of vibrissal follicles of the rat, rabbit, and guinea pig, and in wool roots treated with 8 M urea to remove the unkeratinized parts of the root (FIGURE 5). It is reasonable to suggest therefore that this amino acid is generally present in inner root sheaths of hair follicles. However, there remains the important question of whether the citrulline is bound covalently to the protein by peptide linkages or whether it is free citrulline strongly adsorbed to the protein. Even

if the latter alternative were true the result would be interesting in view of the large amount of citrulline present and the tenacity with which it is held.

Inner root sheaths have been soaked for long periods in changes of 8 M urea, 98 to 100 per cent formic acid, 0.1 N HCl, 0.1 M EDTA, and 0.9 per cent saline but with no significant decrease in the intensity of the chromatogram spot. If the citrulline were bound by hydrogen bonds, for example involving the ureido group (FIGURE 15), 8 M urea or 98 to 100 per cent formic acid would be expected to release the amino acid. Similarly HCl and EDTA should have disrupted any binding by salt linkages or by chelation. Moreover, these extractions should have sufficed to remove small peptides or proteins containing citrulline. Two further pertinent observations are the absence of DNP-citrulline in hydrolyzates of inner root sheaths treated with FDNB—if free citrulline were present its DNP derivative should have been detected—and the absence of a citrulline spot on chromatograms of hydrolyzates of the roots of the hairs from which the inner root sheaths had been dissected.

One is lead to conclude, therefore, that the citrulline in the inner root sheath is combined in peptide linkage in the major component of the sheath, the fibrous protein. The only other recent claim seen in the literature of the occurrence of citrulline in a protein was in a report of investigations on the alga *Chondrus crispus*[34] but later work[35] has shown that it was combined in this organism in the form of small peptides and not in the insoluble protein itself.

The unusual finding of citrulline in the inner root sheath necessitates further confirmation with experiments to demonstrate its presence in peptide linkage in the fibrous protein. These experiments would test:

(1) The mechanical disruption of the cells and exhaustive extraction of the nonprotein nitrogen from the homogenised cells followed by analysis of the residue, or the extraction of the protein from the inner root sheath and analysis of this.

(2) The determination of the extent of binding of citrulline by the inner root sheath using citrulline labeled with C^{14}.

(3) The isolation of peptides containing citrulline from partial hydrolyzates of the inner root sheath or the isolated protein.

(4) The detection of citrulline in the inner root sheath by a histochemical method. The ureido group should provide a suitable reactive site for a specific histochemical method.

Very little can be said at this stage about the function of citrulline in the inner root sheath. It is not known whether citrulline combined in a protein can participate in biochemical reactions, but it has been suggested[12,13] that citrulline in the inner root sheath could be derived from arginine side chains by an enzyme-catalyzed reaction in which the imino group is removed as NH_3. This NH_3 might be utilized by the growing hair to amidate the acidic side chains in the proteins of the developing keratin. An enzyme described by Szörenyi *et al.*[36] acts on free arginine to give citrulline (FIGURE 15), but preliminary experiments using wool roots[11] have not indicated the presence of this enzyme. Such a conversion of arginine side chains into citrulline rather than direct incorporation of the amino acid would accord with electron microscopic evidence[29] suggesting that trichohyalin granules are converted into the fibrous protein. These granules and the fibrous protein are apparently two forms of the same

protein. Experiments have indicated that trichohyalin-granules extracted with 8 M urea contain little or no citrulline, and it is probable therefore that their conversion to filaments is accompanied by chemical changes as well.

Summary of the Structure and Composition of Proteins in the Hair Follicle

Figure 16 is a diagrammatic representation of the structure and composition of several layers in a hair follicle. The information summarized in it is derived from the numerous studies of the cortex, of the cuticle, and of the inner root sheath discussed in this and a related paper (Rogers[37]) together with the recent results of Bradbury[38] on the composition of the cuticle, and those of Matoltsy[39] on the medulla.*

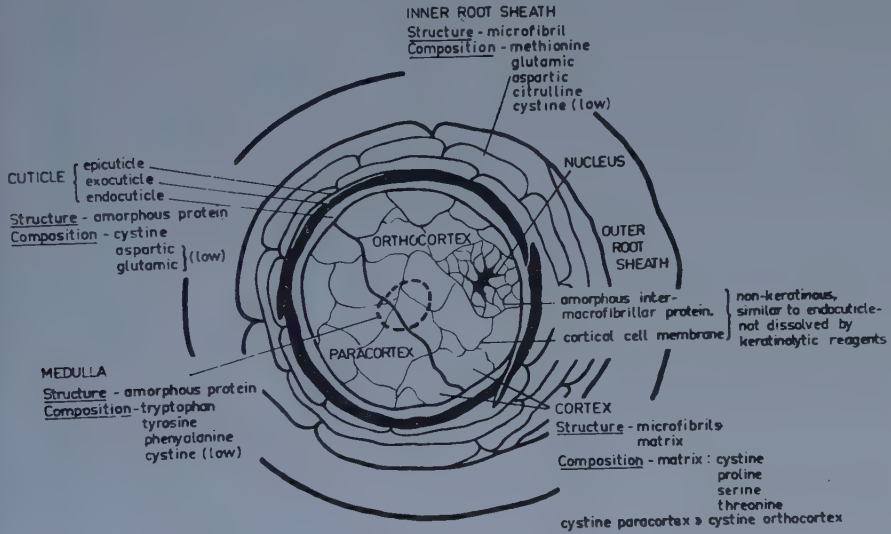

Figure 16.

The diagram particularly refers to a wool follicle in which the fiber has a bilateral structure, as in the merino wool fiber, and is situated eccentrically in the follicle. The medulla is absent from wool follicles of fine-wooled animals, but it is present in larger hairs, so its location is indicated. Except where other-

* The results of more recent studies of the protein of medullary cells from rabbit hair and porcupine quill (Rogers and Rowlands, to be published) do not entirely agree with those of Matoltsy. Certainly the tryptophan content is somewhat higher than in the keratin of the cortex but the contents of the other aromatic amino acids are not significantly different. Only traces of cystine were detected on paper chromatograms of acid hydrolyzates of medullary cells, and none could be detected in the quantitative amino acid analyses using the Moore and Stein technique. The cystine may have come from contamination by traces of cortical keratin.

Of particular interest is the fact that the amino acid composition of the medullary protein resembles that of the protein of the inner root sheath. More than one half of the side chains of the medullary protein are those of the acidic and basic amino acids, particularly lysine and glutamic acid. It is, therefore, a highly charged protein. Moreover, the amino acid citrulline has also been found to occur in significant amounts. As in the inner root sheath it appears that the citrulline is a constituent of the medullary protein and is not free.

wise shown, the amino acids mentioned for each component are those present to a greater extent than in whole hair or, in this case, in wool.

Acknowledgments

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Part II. Pattern Alopecia

A MALE PATTERN BALDNESS IN WATTLED STARLINGS RESEMBLING THE CONDITION IN MAN*

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Several species of animals have been considered with regard to usefulness in studies of male pattern baldness (MPB). Baldness of this type occurs in primates such as the ouakari (Walker, 1956), but procurement of these animals is costly and their care difficult.

Among mammalian species commonly reared in the laboratory, mice have been reported (Kochakian, 1940) to exhibit a sparse piliary coat after androgenic treatment. The condition produced in mice differed from MPB in several respects, however; the characteristic patterns were absent, the alopecia did not spread from an affected region to adjacent areas, and the state was not elicited in females. In another species, the guinea pig, the number of hairs per square centimeter of perianal skin becomes reduced following sexual maturation; but tattoos placed in the dermis to identify the individual hair follicles demonstrated that the change after maturation represented an expansion of "sex skin," not a loss of hairs.

Certain species of birds, which might be kept in the laboratory, acquire baldness of the scalp as a result of what appears to be androgenic stimulation. Examination of preserved specimens of many species in the American Museum of Natural History, New York, N. Y., showed that MPB was developed to a pronounced degree by the wattled starling, *Creatophora carunculata*, and to a more limited extent by the sandpiper, *Philomachus pugnax*, as described for the latter species by van Oordt and Junge (1936). Descriptions of wattled starlings and their changes in plumage are given in the text and references by Chapin (1954).

The wattled starling was selected as an experimental animal, and the similarities and dissimilarities of MPB in this bird and man were examined at length (TABLES 1 and 2), since counterparts of human disease in animals require critical scrutiny. The similarities between the two species are impressive, especially with regard to the mechanisms responsible for MPB, and augur the value of wattled starlings as experimental animals in studies of this form of alopecia and in formulation of therapeutic procedures. The outstanding similarity is that androgenic stimulation interferes in local areas with normal replacement of integumentary appendages that have a finite lifespan, that is, appendages that are ordinarily lost and regenerated at intervals.

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Results

Seasonal Changes in Intact, Untreated Starlings and the Regeneration of Feathers After Plucking

Males in their first year of captivity. The occurrence and extent of seasonal changes varied considerably in individual birds. Nuptial plumage, accompanied by MPB of the head (FIGURE 1*a* to *d*), was exhibited by 60 per cent of

TABLE 1
SIMILARITIES OF MALE PATTERN BALDNESS (MPB) BETWEEN WATTLED STARLINGS AND MAN

(1) Structures under consideration are integumentary appendages that have a finite lifespan and are replaced at intervals.
(2) The process under consideration is inadequate replacement of this type of integumentary appendages.
(3) MPB is limited to the head. The skin of the head exhibits regional differences in susceptibility to MPB, which progresses from one area to another in a bilaterally-symmetrical fashion.
(4) Androgens are the usual incitants of MPB. Fully-developed alopecia is common among adult males but is not acquired by most normal females. MPB is absent in the immature and does not develop in males after castration.
(5) Androgens incite MPB in castrate, immature, or female subjects. Alopecia develops to the fullest extent only after prolonged androgenic stimulation, when tested in young individuals.

TABLE 2
DISSIMILARITIES OF MALE PATTERN BALDNESS (MPB) BETWEEN WATTLED STARLINGS AND MAN

(1) Structures under consideration are feathers versus hair.
(2) In birds, but not in man, the process under consideration is reversible in intact subjects. Some regions are refeathered as a normal event after each breeding season.
(3) The baldness-inciting testicular secretions are produced chiefly during the breeding season in birds but throughout the year in man.
(4) Genetic predisposition to MPB upon androgenic stimulation has been exhibited by all birds tested thus far but is not present in all men and women.
(5) In birds the areas subject to MPB exhibit peculiarities. There is a pale region posterior and superior to the eyes. The skin becomes redundant, corrugated and, in some sites, melanotic or orange in color.
(6) In birds, MPB and refeathering bear relationships to body-wide moulting and seasonal plumage.
(7) In bald areas the integumentary appendages tend to be absent in birds, whereas they are present but reduced in size in man.

the males (12 of 20) with the advent of the breeding season in spring or late winter. Birds that became bald remained so during the rest of the breeding season (FIGURE 1*a* to *d*).*

The period of the year when the scalp was bald was variable (FIGURE 8) among birds maintained under comparable circumstances in cages (12 ft. × 7 ft. × 4 ft.) that permitted short flights. Greater uniformity might be obtained with less confinement, a better diet, the use of birds of known age, and

* Letters in the lower right corners of all illustrations in this article identify the sequence of figures in a plate. The month and day that each photograph was taken are shown in every photograph. Squares in the backgrounds are 1 cm. across.

longer acclimatization, but even in the same starling the dates of acquisition and loss of nuptial plumage differed by a month or more in successive years (FIGURE 8).

In summer or autumn the nuptial status was replaced by the eclipse feathering (FIGURE 1*e*) characteristic of the nonbreeding season. This change was associated with fading of melanin of the skin and bill (FIGURE 2, compare *g* with *i*).

To ascertain the capacity for replacement of feathers, one side of the head was plucked repeatedly in 6 males. The capacity for refeathering varied with the season of the year, the region of the skin and the individual bird. Generally, regrowth was rapid and complete in the nonbreeding season, absent or incomplete during the breeding season. Failure to refeather plucked areas during the breeding season was noted even in males that did not become bald (FIGURE 3*c* to *e*) on the unplucked side of the head. These findings are exemplified by a nonbald bird that refeathered rapidly after each of 4 pluckings between September and April; after the next plucking in May the feathers failed to regrow in the region posterior, and to some extent superior, to the eyes. This region, which is particularly susceptible to baldness, is referred to hereafter as paraocular (PO).

At no time of the year did the impetus to refeathering, which is afforded by plucking, induce growth of feathers in nearby bald regions.

Females in their first year of captivity. No instance of fully developed MPB was observed in 18 untreated females. Many birds developed increasingly large PO bare areas during the breeding season, however, and tended to refeather these areas to some degree during the nonbreeding season.

Repeated plucking of one side of the head in 5 females showed that regeneration of feathers proceeded rapidly in the nonbreeding season but did not occur during the breeding season in 3 of the 5 birds.

Regional sequence of changes in males during the breeding and nonbreeding seasons. The following regions are listed in a commonly observed sequence of increasing threshold and delay in the development of baldness: (1) the PO area that increases in size posteriorly and superiorly; (2) the midline of the scalp superior to the posterior portion of the eyes; (3) the region anterior and anterodorsal to the eye; (4) the midline of the scalp anterior to the pigmented coronal band; and (5) the skin adjacent to the ear and along the anterior border of the pigmented coronal band.

During the breeding season a few males acquired only PO denudation (FIGURE 3) but most males retained feathers chiefly in tracts along the mid-dorsal scalp, occasionally as an uninterrupted strip (FIGURE 2*e*), or as clusters at both ends (FIGURE 1*c* and *d*); in advanced cases the strip was interrupted and the anterior and posterior groups reduced to isolated feathers (FIGURE 1*f*). In a few males the auricles became visible (FIGURE 1*d*) and, in rare instances, were almost completely exposed.

The order of changes with the onset of the breeding season was reversed at the end of the season, that is, the effects that had been first to appear tended to be the last to disappear. At the end of the breeding season a similar sequence also characterized females that had been plucked during the breeding season (FIGURE 4*f* to *i*).

DORSAL 5-19
1 CM.
a
DORSAL 7-13
b
11-12
e
5-19
c
7-13
d
DORSAL 5-19
f
5-19
g
VENTRAL 5-19
h

FIGURE 1.

Incidence of MPB in wild starlings. Because of death from water deprivation during transit, 2 groups of starlings were autopsied a few days after collection during 1954 in Nanyuki, Kenya, Africa which is near the equator. Of 21 males trapped in March only 29 per cent were bald, whereas 2 months later 64 per cent of 25 males were in nuptial plumage and bald. This incidence of MPB during the breeding season may be compared with that of 60 per cent among males in their first year of captivity.

Among females there were no bald birds in 12 collected in March and only 2 instances of extensive loss of feathers among 9 trapped in May. One of these 2 birds had bare areas, superior to the eyes, which merged across the midline of the scalp over an anteroposterior distance of 0.3 cm.; the other female retained only a thin band of feathers along the midline of the scalp. These were definite, although borderline, instances of MPB according to criteria employed in this study.

The extent of the bare areas of the scalp was not closely correlated with the size of the testes or ovaries.

Prevention of MPB by Castration

In 15 males the testes were removed bilaterally without puncture of the tunica albuginea. Apparently, no testicular tissue remained or regenerated after orchiectomy since, thereafter, (1) none of the birds became melanotic or exhibited the growth of wattles and comb characteristic of androgenic stimulation or the breeding season, and (2) no testicular tissue was detected at autopsy.

Three castrates were treated with androgens as described in a later section of this paper. The other 12 castrates were left untreated and caged with 10 intact female and 10 intact male controls (8 of which were mock-castrated).

Nine of the 12 untreated castrates had feathered scalps at the time of orchiectomy and none of them became bald subsequently at the time of the breeding season. The lack of MPB in any of these birds is attributable to absence of the testes since (1) 6 of these birds had been bald in the breeding season of the previous year (FIGURE 2*b*); and (2) MPB occurred during the ensuing breeding season in 70 per cent of the 10 intact control males. Mere operative procedures can be excluded as a factor, since 5 of the bald control males had been mock-castrated in that the testes had been removed and reimplanted (FIGURE 2*b*).

Three of the fully-feathered, untreated castrates were left untreated for a second year and failed to become bald during the second as well as the first

FIGURE 1. Two intact males with extensive development of male pattern baldness (MPB) during the breeding season. Feathers were not plucked. Male No. 3(*a* to *e*,) note that MPB and other changes characteristic of breeding seasons were maintained for several months as shown in *a* to *d*. Only a few feathers remained in certain sites such as the region rostral to the eyes, the lateral aspects of the lower jaw, and an anteriorly-directed wedge-shaped area at the posterior of the midline of the scalp. By July (*d*) the auricle was exposed; baldness to this extent was unusual. Melanotic pigmentation was extensive on the anterior part of the head. A coronal band of pigment, superior to and surrounding the auricle, was partially hidden by overlying feathers (*d*). Comb and wattles were pigmented and proturberant (*d*). There was a large pale area with a baggy posterior border (*b*). By November (*e*) this bird was in the nonbreeding season and had refeathered except for a large area adjacent to the eye. For 5 days before this photo was taken, the bird had been treated with androgen; pigmentation but not denudation had developed. Male No. 18(*f* to *h*), this male was bald and lacked the wedge-shaped area of feathers at the posterior of the midline although he retained isolated feathers in sites that usually remain sparsely feathered.

DORSAL 5-18
a
DORSAL 6-3
b
5-18
c
6-3
d
5-18
e
8-9
f
5-19
g
DORSAL 7-13
h
11-2
i

FIGURE 2.

breeding season. Both years the castrates tended to acquire slightly enlarged areas of PO denudation at the time of the breeding season.

One side of the head was plucked repeatedly in all 3 of the fully feathered, untreated castrates. Feathers regrew readily throughout the nonbreeding season but not during the time of the breeding season (FIGURE 5*b* and *c*).

In 3 males castration was deferred until the breeding season when the birds were already bald. Refeathering did not occur until the end of the breeding season.

It may be concluded that in castrate as well as intact birds the regeneration of feathers after plucking tended to be more inhibited during the time of the breeding season than thereafter. Gonadal secretions could not be responsible for this phenomenon in castrate birds.

Effects of Exogenous Androgens and Estrogens

Since baldness did not occur during captivity in castrate males or intact females, or in intact males during the nonbreeding season, these categories of wattled starlings can be used to test agents and circumstances that promote MPB. Accordingly, 13 fully-feathered birds (3 castrate males, 6 intact females, and 4 intact males in the nonbreeding season) were treated with androgens or estrogens, given by different routes for various lengths of time. The same birds were used successively in several experiments.

Findings may be summarized as follows:

(1) Androgens induced MPB in all birds of either sex. The production of MPB in castrate males is proof that this effect need not be mediated indirectly through the agency of the testes.

(2) The regional progression of MPB upon stimulation by exogenous androgens resembled that in intact males upon the onset of the breeding season. Small dosages of androgen incited only early phases of the sequence. Intensive and prolonged treatment produced in either sex more extensive MPB (FIGURE 7*b*) than occurred normally in intact animals.

(3) MPB occurred upon treatment with testosterone, androsterone or dehydroisoandrosterone. This suggests that MPB can be elicited by different androgens and is not a response restricted to testosterone alone.

(4) Once produced, MPB remained as long as exogenous androgens were administered.

FIGURE 2. Three intact males that developed MPB during the breeding season but retained a strip of feathers along the midline of the scalp. Feathers were plucked on the right side of males 5 and 7. Male No. 5(*a* to *f*), during the breeding season feathers did not regenerate after plucking; this suggests that the feathers on the unplucked left side (*e*) were retained rather than regrown. Note increased pigmentation and comb growth in the breeding season (*a* versus *b*). The testes that had been removed and reimplanted in April, produced these seasonal changes, demonstrating that operative trauma does not account for the prevention of baldness by castration. Unplucked male No. 6(*g* and *h*), retention of feathers along the midline of the scalp in the early part of the breeding season (*g*), only isolated feathers by July (*h*). This bird refeathered after the breeding season and was castrated the next spring. During the time of the next breeding season after castration, denudation was limited to a paraocular region comparable to that in FIGURE 5*e*. Male No. 7(*i*), the small amount of melanin in the nonbreeding season is shown after plucking of the right side of the head and neck. The anterior comb was still pigmented but had shrunk. Refeathering (after baldness during the breeding season) was still proceeding as shown by pinfeathers at the fringe of the pale area, a region that is late to refeather and remains bare in some birds.

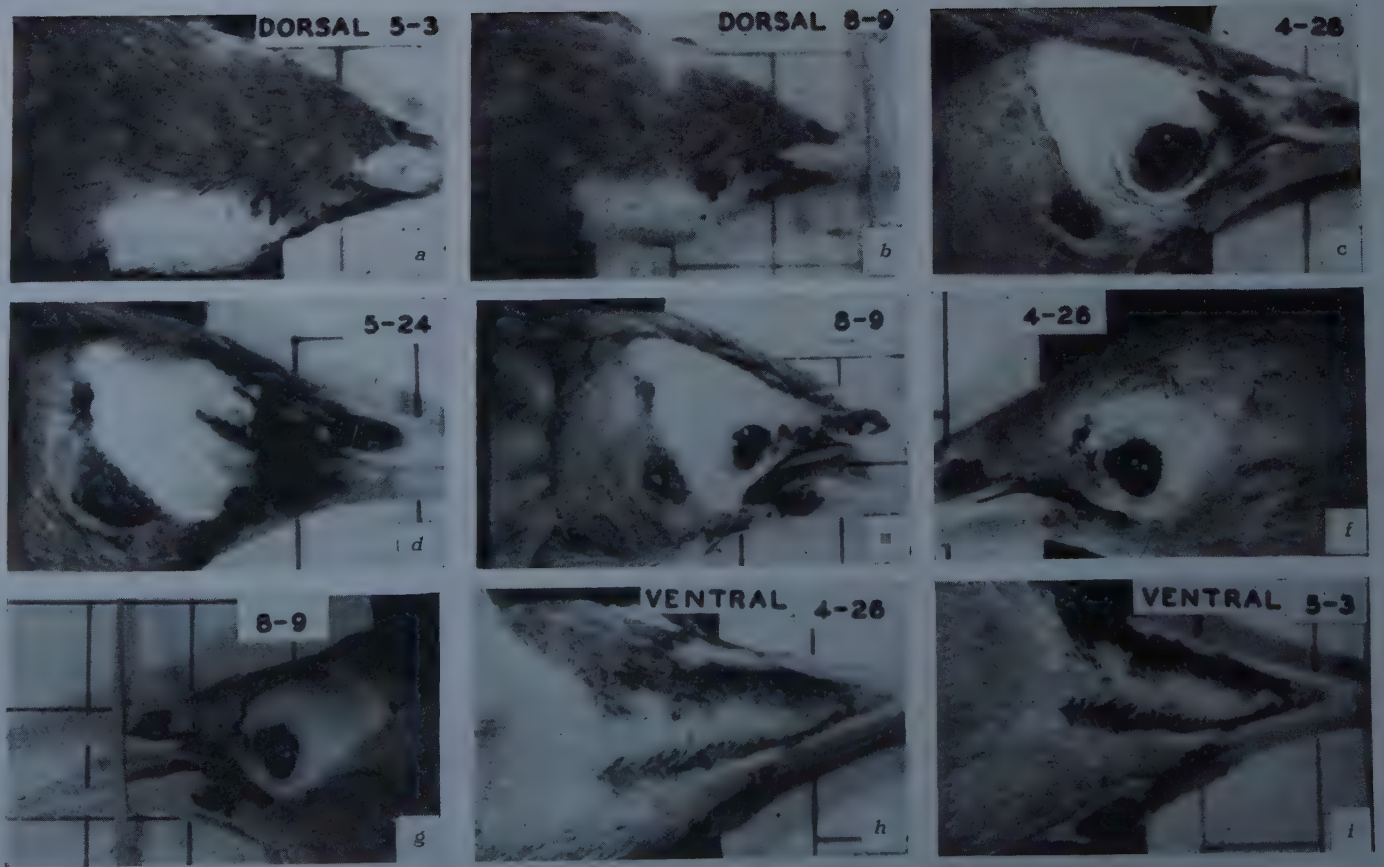

FIGURE 3. Intact bird No. 12, classified as a male on the basis of plumage. Only the paraocular area became bare during the breeding season (*f* versus *g* and *a* versus *b*), although pigmentation increased markedly as shown on the right side (*c* versus *d*), which was plucked and did not refeather during the breeding season. The wattles became pigmented, but only slightly enlarged (*h* versus *i*) and the combs remained small. Pigmentation decreased toward the close of the season (*e*).

FIGURE 4. Intact female No. 13 acquired only slight paraocular denudation on the unplucked left side (*b* versus *c*) during the breeding season but developed considerable melanin as shown on the plucked right side. Wattles became pigmented but only slightly pendant (*e*). Combs remained vestigial. The regional sequence in refeathering is shown in *f* to *i*. Feathers regrew earliest on areas that had been the last to become bald (*f*)

FIGURE 5. Four males, castrated when feathered, that did not develop MPB thereafter during the breeding season; and one male (*g* to *i*) that was already bald when castrated. Male No. 10, feathered when castrated in April; at the time of the breeding season the bird never became bald (*a*) and regenerated few feathers on the plucked right side (*b* and *c*). The fading of pigment and the thin atrophic anterior comb are shown 40 days after castration (*c*). Male No. 8 (*d*) had a thin strip of feathers in the midline of the scalp when castrated in April. Neither the plucked right side of the head nor the unplucked left side showed any change in feathering until after the breeding season. Males No. 9 (*e*) and No. 14 (*f*) had large bare paraocular areas when castrated in May. These bare areas became slightly larger at the time of the breeding season but a thick strip of feathers was retained in the midline. The birds were not plucked. Male No. 15 was bald and extensively pigmented when castrated (*g*, *h*). Near the auricle and the bill a few feathers regrew sporadically by the end of June, but the surge of refeathering in bald regions did not occur until early September in the now-pale bird (*h*).

FIGURE 6. A male, bald when castrated, that did not refeather until after the breeding season; also a feathered female that became minimally bald upon treatment with small doses of androgens. Male No. 16 (*a* to *f*), castrated in April when bald except along the posterior midline of the scalp, soon showed the fading of melanin (*a* with right side plucked, left side unplucked). Weekly examination showed that feathers were not replaced until after the breeding season (*b*, *d*, and *f*); then the head became extensively covered except paraocularly. In female No. 17 the left side of head had been plucked and inuncted daily with 5 gamma of testosterone, beginning in October (*g* of the untreated state). This dosage of androgen produced and maintained a limited but definite degree of MPB with feathers remaining in areas somewhat resistant to alopecia such as around the auricle (*h*), on the anterior scalp (*h*, *i*), and along the dorsal midline (*i*). Discontinuation of inunction after 5 months was followed by extensive growth of feathers except in paraocular areas.

DORSAL 12-12-52
a
DORSAL 3-5
b
4-5-53
c
10-22
d
DORSAL 10-22
e
12-12
g
DORSAL 12-12
f

FIGURE 7.

(5) Repeated courses of androgenic treatment in the same bird invariably resulted in MPB.

(6) Androgenic treatment interfered with the replacement of feathers to such an extent that, after prolonged intensive treatment, feathers were absent in most of the follicles in bald areas of skin.

(7) Some degree of refeathering occurred following the withdrawal of androgen, even though stimulation had been prolonged or the bird had been subjected previously to many periods of treatment.

(8) MPB was prevented in intact adult males by the administration of certain compounds including estrogenic steroids.

The above findings are illustrated by the following protocols:

The longest periods of uninterrupted androgenic treatment were obtained with twice-monthly subcutaneous implantation of 2 pellets, each containing 10 mg. of testosterone propionate. Treatment was begun in October and continued for 4 months in 1 male, 9 months in 1 female, and 13 months in another female. Feathers were plucked from the right side of the head when the pellets were implanted. On the unplucked side, feathers became sparse within 2 months. Subsequently the remaining feathers were lost and none regrew on either side of the head. In all 3 birds MPB remained as long as androgen was administered. FIGURE 7*c* shows an example.

Two females were subjected to 5 alternate periods with and without implantation of androgenic pellets. MPB developed during each period of stimulation, and refeathering occurred each time that androgen was withdrawn (FIGURE 7*b*).

Two females known to develop MPB in response to implanted pellets of testosterone propionate were utilized in preliminary explorations with less intensive androgenic stimulation and topical applications to the scalp. Beginning in June, when both birds were feathered, 0.5 mg. of testosterone propionate in 0.05 cc. of peanut oil was injected subcutaneously each week, to 1 bird in a single dose, to the other in subdivided doses given Mondays and Thursdays. The right side of the head was plucked on the first day of injection. Injections were continued for 6 weeks in one bird, 36 weeks in the other, during which time the plucked side remained bare. The unplucked side acquired slightly larger areas of PO baldness by the sixth week in both birds and later became bald in one bird.

In October of another year, feathers were plucked from one side of the head

FIGURE 7. Female No. 2 (*a* to *c*), fully-feathered in October of 1952, when the right side of the head was plucked and the bird given the first of twice-monthly implantations of 2 pellets of testosterone propionate. By December (*a*) feathers had not returned on the plucked right side and only scattered ones remained on the unplucked left side; despite the sex of the animal and the season of the year, pigmentation was more extensive than observed in any intact male during the height of the breeding season. After 5 to 6 months, continued androgenic treatment produced still more extensive baldness and pigmentation (*b* and *c*); note the bare auricles, wattles, and combs. All remnants of pellets of testosterone propionate were removed in March of 1953 because of the scrawny appearance of the bird (*c*) and, within a few weeks, refeathering was complete except paraocularly. Female No. 4 (*d* to *g*). The lateralmost portion of the right side of the head had been plucked and the bird subjected to androgenic stimulation beginning 4 days before *d* and *e* of the fully feathered state in October. By December (*f* and *g*) pigmentation was intense, wattles and combs hypertrophied. Feathers had become sparse on unplucked areas (*g*) and did not regenerate in the region previously plucked (*f*). Subsequently the entire scalp became bald and remained so while androgenic treatment was continued for 13 months.

of a female; this bird had been unable to refeather after plucking when 0.25 mg. of testosterone propionate had been injected twice weekly. One side of the scalp (near the bill) was inuncted daily with 5 gamma of androgen in 0.02 cc. of ethanol. Melanotic pigmentation appeared in the usual areas and feathers became sparse on regions most susceptible to MPB (FIGURE 6*h* and *i*). Systemic effects were implicated since the MPB was bilaterally symmetrical. FIGURE 6*i* shows that even after 19 weeks of treatment both the unplucked and plucked sides were feathered on regions with high thresholds for androgen-induced MPB: for example, around the ears and on the anterior portion and midline of the scalp. Therefore the dosage of androgen was in excess of that

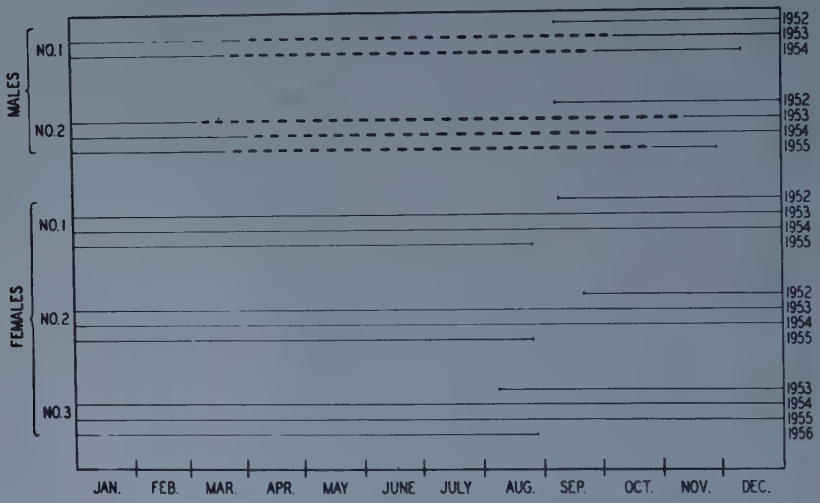

FIGURE 8. The *dash line* represents baldness of the scalp, the *uninterrupted line* the fully feathered scalp. The data are for birds that were not used in experimental studies and survived the longest. In the laboratory the development and duration of baldness, in association with the breeding season, occurred only in males and varied considerably from animal to animal and, in successive years, in the same bird.

required to incite a minimal degree of MPB but insufficient to produce MPB to the extent present physiologically in most intact males (FIGURE 6*h*).

Implantation twice monthly of 2 pellets of androsterone in one female, and of dehydroisoandrosterone in another female, beginning in October, resulted in MPB and pigmentation similar in nature and in time of occurrence to the changes produced by testosterone.

After MPB from temporary treatment with any androgen, regions with a high threshold to baldness became almost completely refeathered in all of eleven birds studied in this regard. Areas less resistant to baldness refeathered to different extents in various birds. Regions highly susceptible to baldness, such as the PO, were usually not re-covered by feathers (FIGURE 6*g*).

Immediately prior to the breeding season, 2 males (which had been bald the previous summer) were subjected to prolonged estrogenic treatment afforded by twice-monthly subcutaneous implantations of a 10 mg. pellet containing 1

part of estrone and 3 parts of cholesterol. Neither animal became bald or melanotic during the ensuing breeding season.

Estrogenic treatment of this type, given to 2 bald males in the breeding season, resulted in a fading of most of the grossly-evident melanin within 24 days, but had not induced refeathering of the bald areas when the experiment was terminated after 3 months.

Melanin

Melanotic pigmentation was related to the endocrine status and reflected androgenic stimulation. Pigmentation was minor in both sexes during the nonbreeding season. It became greatly intensified in males during the breeding season (FIGURE 3*c* and *d*), reaching the extent shown in FIGURE 3*e*. Birds that became bald always developed extensive melanin that was maintained during the breeding season (FIGURES 1*a* to *d* and 2*a* and *b*). Males that did not become bald also exhibited some degree of melanism during the breeding season; this can be seen after plucking (FIGURE 3).

Castrate males did not acquire melanin during the breeding season and lost pigmentation if melanin were present at orchiectomy (FIGURE 5*d*). Estrogenic treatment of intact males prevented development of melanin during the time of the breeding season and produced a marked loss of pigmentation when administered to males in the nuptial state.

In females melanin increased during the breeding season in many of the same integumental areas as in males (FIGURE 4*a* and *b*). The amount of melanin varied from female to female but tended to be considerably less than in bald males (FIGURE 1*c* and *g*) and even less than in nonbald (FIGURE 3*d*) males.

Pigmentation in excess of that observed in untreated normal animals was produced by intensive treatment of either sex with exogenous androgens (FIGURE 7*b*). Withdrawal of androgenic treatment led to progressive disappearance of melanin until it was restricted to a thin line along the opposing margins of the proximal parts of the bills; this occurred at any season in castrates and during the nonbreeding season in intact males and females.

DISCUSSION

Endocrine effects upon plumage. Comparisons of different species (Boss, 1943) have shown that sex differences in plumage are controlled by a variety of mechanisms: (1) by heredity as in finches, especially the English sparrow; (2) by interactions between genetic and endocrine factors as in pheasants; and (3) by endocrine regulation in one sex whereas in the other sex the plumage is "neutral," that is, unaffected by secretions of testes or ovaries. In domestic fowl, henny plumage is induced by estrogens and cock plumage is neutral. In herring gulls, cock plumage is regulated by endocrine factors. In wattled starlings the present data show that baldness of the scalp, which is expressed most fully in the nuptial plumage of males, is incited by androgens.

Processes involved in androgenic induction of baldness. In starlings the bare appearance of the scalp was found to be due not to mere expansion of the skin between adjacent follicles but to failure in replacement of feathers. This failure was so complete as (1) to affect almost all follicles in certain regions of

the head and (2) to be continued without interruption during androgenic treatment given for thirteen months. In general the few feathers that remained in certain alopecic areas seemed to represent retained feathers, since regeneration after plucking was limited both at the time of the breeding season and during androgenic treatment.

In man the number of hairs per square centimeter of scalp seems to be similar in bald and nonbald areas (Hamilton, 1959). Therefore in man the follicles in bald areas continue to produce hairs, but these hairs are tiny. Van Scott and Ekel (1958) have shown that the dermal papillae as well as the hair bulbs are reduced in size in bald regions.

However, in both the wattled starling and man, MPB seems to be a result of androgenic interference (Hamilton, 1942) with normal replacement of integumentary appendages that have a finite life span, that is, must be regenerated at intervals. Periodic replacement also characterizes other structures with cyclic growth related to testicular secretions; examples are the antlers of deer and horns of prong-horned antelopes. There are specificities, however, as well as generalities with regard to androgenic interference with replacement of appendages. In man, for example, androgens inhibit regeneration of hair in certain but not in all areas of the scalp, and do not prevent replacement of hairs in the beard or of nails that have been removed.

There is evidence that androgens not only interfere with replacement but may also shorten the period of growth of certain of the structures that ordinarily have a finite life span. Androgenic stimulation not only prevents regeneration of the hairy skin that sustains the growth of deer antlers but results in loss of this skin after it has grown (Wislocki *et al.*, 1947). The possibility that androgens reduce the period of growth of feathers and hairs in baldness-susceptible areas deserves investigation. It is known that hairs have a shorter life span and tend to grow faster in men than in women (Myers and Hamilton, 1951). This phenomenon was observed not only on the tonsure but also in the suprarear region of the scalp; the latter site is not susceptible to baldness.

There is a possibility that MPB is a consequence of androgen-promoted differentiation of certain cells. Differentiation is a form of specialization commonly associated with a reduced capacity to form new cells and structures; this is true, for example, in hemopoiesis, spermatogenesis, and keratinization. The incitation of MPB by the furthering of differentiation would be in harmony with known actions of androgens in promoting prenatal (Burns, 1955) and postnatal growth and specialized function of male secondary sex organs and characters. Postnatal stimulation by androgens has been described for prostate and other secondary glands of the male reproductive tract (Moore, 1939); sebaceous glands (Hamilton, 1941, Hamilton and Montagna, 1950); nails in male lizards (Evans, 1951); melanin-producing cells in the integument of many species (see Hamilton and Montagna, 1950); and presumably is involved in the growth of horns in prong-horned antelopes (Caton, 1881).

Induction of MPB in birds argues strongly against claims (Flesch, 1951) that an important or necessary role in androgen-induced baldness is played by squalene and other elements in the sebum produced by pilosebaceous apparatus. There are no sebaceous glands in the scalp of these birds.

The capacity to respond to androgen by baldness resides in the integumental areas subject to MPB. From studies in man it was concluded that "the capacity and character of the reaction (baldness) is in some large part an inherited property of the cells themselves" (Hamilton, 1942). This conclusion is supported by studies (Hamilton, 1959) of 2 wattled starlings in which autografts were exchanged between baldness-susceptible areas of the scalp and baldness-resistant skin of the neck. The transplants retained properties possessed in their original sites in that their production of feathers was similar to that in symmetrical areas that were not grafted. For example, feather germs from baldness-susceptible areas, when transplanted elsewhere, continued to respond to androgens by failure to produce feathers.

The following previously unreported observations in man further substantiate this conclusion.

I had the opportunity to observe twelve men in whom small autografts of the scalp were used to replace skin of the head that had been damaged in accidents and wounds incurred during World War II. In eight men, an already-bald scalp was transplanted to facial areas in which coarse hairs were undesirable. In two subjects, hairy skin from the side of the head was transferred to regions of the scalp symmetrical with areas that were bald. In all cases the autografts continued to behave as they had before transplantation.

Perhaps even more crucial circumstances obtained in two other young men in whom scalp from baldness-susceptible areas, which still bore hair, was transferred as autografts. One of these men reported that, at first, hair had grown on the graft but that it was lost at a later time when the ungrafted symmetrical area became bald; photographic evidence was suggestive but not documentary. The inference in this case led me to carry out serial studies of a man whose autografted scalp was still hairy. The man was disfigured and unwilling to permit periodic photography, but inspection was possible and clearly demonstrated that baldness progressed almost simultaneously on the graft and on the symmetrical area of the scalp *in situ*. The graft had been taken from the frontoparietal scalp, a region susceptible to early stages of MPB.

Seemingly in harmony with the above observations in starlings and men is the convincing demonstration by Orentreich in a companion article in this publication that donor, rather than recipient, "dominance" characterizes autografts exchanged between bald and nonbald areas in men with MPB.

Genetic susceptibility to MPB. The spontaneous occurrence of MPB in many but not all intact male starlings might seem to be analogous to the occurrence of MPB in most but not all intact men. The information at hand indicates, however, that genetic susceptibility to MPB is more universal in starlings than in man. All thirteen of the birds that received prolonged intensive treatment became bald. Furthermore, untreated females tended to acquire more extensive MPB in starlings than in the human species.

Summary

The following observations were derived from laboratory studies of the wattled starling, *Creatophora carunculata*.

The majority of adult males developed a male pattern baldness (MPB)

during the breeding season. After the breeding season the head became refeathered with the exception of regions posterior and superior to the eyes.

MPB did not develop in females, in castrate males, or in what appeared to be young males, although some of the birds exhibited paraocular denudation at the time of the breeding season.

Upon administration of exogenous androgens all birds of either sex, whether castrate or intact, acquired MPB that persisted as long as treatment was continued (maximum of 13 months). Prolonged and intensive androgenic stimulation induced MPB and melanotic pigmentation in excess of that in untreated intact animals.

Refeathering occurred upon the withdrawal of androgen in all birds, even after continuous treatment for as long as nine months.

Various regions of the skin of the head were found to differ in susceptibility to MPB. The sequence of changes upon androgenic stimulation was reversed upon cessation of the stimulation, that is, the phenomena that had been the first to appear tended to be the last to disappear.

Conclusions

Animals of the species *Creatophora carunculata* have the capacity to develop a male pattern baldness (MPB) that resembles in several basic features the common form of baldness in man.

Castrate males, intact males in the nonbreeding season, and females can be employed for testing of conditions and treatments that incite MPB, for experimental analyses of the processes concerned and for screening of agents useful in prophylaxis and treatment.

In essence, MPB seems to be an androgen-induced interference, in local areas, with the normal replacement of integumental appendages.

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VASCULAR PATTERNS ASSOCIATED WITH CATAGEN HAIR FOLLICLES IN THE HUMAN SCALP*

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Introduction

The growing (anagen) hair follicle is richly supplied with blood vessels. In addition to the capillary loops within the mesodermal papilla, a plexus of vessels is embedded in the connective tissue sheath surrounding the lower third of the follicle and an upper plexus encircles the pilosebaceous canal.[1,2] In the quiescent (telogen) follicle the dermal papilla contains no capillaries and the lower plexus forms a loose bundle of vessels at the base of the club hair, while the upper plexus is unaltered.[1,2] The transitional changes that occur during catagen,[3] the period between anagen and telogen, are not fully known. The present study describes some of the more obvious features of catagen with particular emphasis on the follicular blood vessels. These observations indicate that the degeneration of the blood vessels in the dermal papilla during catagen is a secondary effect and not the primary cause for cessation of hair growth. Changes in the connective tissue sheath, the glassy membrane, and the external root sheath appear first.

Materials and Methods

Autopsy and biopsy specimens of both male and female scalps were stored in dry ice up to 48 hours, and were fixed in cold 10 per cent unbuffered formalin for 4 hours. Sections of the scalp were cut at 30 to 75 μ on the freezing microtome and rinsed in distilled water. They were then placed in a freshly prepared and filtered solution modified from Gomori[4] that contained 10 ml. of a saturated (5 per cent) aqueous solution of sodium borate, 40 ml. cool distilled water, 0.2 ml. of a 1 per cent aqueous solution of magnesium chloride, 40 mg. of sodium alpha naphthyl acid phosphate† and 20 mg. Fast Blue RR†. After incubation at room temperature for 10 min., the sections were placed in fresh substrate solution and left another 10 minutes. The sections were then rinsed in distilled water, transferred to 10 per cent formalin, stored in the refrigerator overnight, and then mounted with glycerine jelly. The endothelium of the smaller blood vessels of the human scalp contains abundant alkaline phosphatase and the deep blue-black reaction product is deposited at these sites. Some sections were counterstained lightly with Mayer's carmalum to reveal the cellular structure of the hair follicles.

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† Dajac Laboratories, Philadelphia, Pa.

Observations

The morphology of the human anagen hair follicle is well known[5-7] and a brief resume is given here merely to clarify the descriptions that follow. Only two components of the follicle contain blood vessels: the connective tissue sheath and the dermal papilla. Both are mesodermal derivatives. The connective tissue sheath is continuous with the papillary layer of the dermis and forms the outermost cellular layer surrounding the follicle. The dermal papilla is almost completely encapsulated within the hair bulb and connects with the connective tissue sheath by a stalk. Within the connective tissue sheath is the noncellular vitreous or glassy membrane that is homologous with the basement membrane of the epidermis.[5,7] This is closely applied to the external root sheath, a cellular epidermal derivative containing abundant glycogen.[6,8] Inside the external root sheath is the internal root sheath, consisting of Henle's and Huxley's layers; within these lie the cuticle and the hair shaft proper.

The essential features of an anagen hair follicle are shown in FIGURE 1. The smooth contours of the connective tissue shealth enclose the transparent glassy membrane. The interface between the glassy membrane and the external root sheath is perfectly straight. The bulb is rounded with its lower portion closely investing the dermal papilla. The dermal papilla contains several capillary loops that pass through the basal stalk of the papilla and connect with the blood vessels embedded in the connective tissue sheath.

The onset of catagen can be detected in human hair follicles by the appearance of characteristic corrugations of the outer root sheath and the concommitant thickening of the glassy membrane and the connective tissue sheath. Similar changes have been observed in the hair follicles of laboratory animals.[2,5,9] The faults in the glassy membrane and the outer root sheath first appear in the lower third of the follicle. The outer root sheath develops one or two corrugations that ring the follicle, either partially or completely. The corrugations are not the result of evagination or contraction of the follicle, but are intimately related to the concurrent thickening of the glassy membrane. During this process, the vitreous layer becomes much more evident than in the normal anagen hair follicle, and the inner surface develops a series of ridges that are mated with the invaginations in the outer root sheath. A space is frequently visible between the interfaces of the two juxtaposed layers; it may, however, be an artifact.

At this point in catagen the follicles apparently have a capillary circulation that is in every respect similar to that of a normal growing hair follicle. Several large parallel vessels originating from larger arterioles in the lower limits of the dermis run longitudinal to the sides of the lower third of a hair follicle forming a palisade. The parallel vessels branch at almost right angles, interconnecting with one another and forming a plexus in the connective tissue sheath. At the lower end of the follicle, several extensions of the longitudinal vessels converge beneath the bulb, enter the dermal papilla, and form several capillary loops.

FIGURE 1*b* shows the lower portion of an early catagen follicle. The blood vessels surrounding the connective tissue sheath, as well as those entering

the dermal papilla in the hair bulb, are patent. One complete capillary loop and fragments of others are shown within the dermal papilla. These vessels show no sign of atrophy. There is considerable destruction of the lower bulb and the outer layer of the upper bulb. The connective tissue sheath, which is outlined by the numerous blood vessels coursing through it, has contracted in the region below and around the bulb and presents an irregular outline. The glassy membrane is much thicker than in the anagen follicle and occupies the wide space separating the connective tissue sheath from the cellular bulb and

FIGURE 1. (*a*) Anagen hair follicle from the human scalp. An arrow points to the blood vessels embedded in the connective tissue sheath. Gomori azo-dye method for alkaline phosphatase, with carmalum counterstain. ×120. (*b*) Early catagen. The connective tissue sheath is slightly constricted at the base of the follicle, there is considerable destruction of the lower bulb and the outer root sheath. The capillary loops within the dermal papilla are patent. Alkaline phosphatase. ×105.

the external root sheath. Several indentations are apparent in the outer root sheath just above the level of the bulb.

Later in catagen (FIGURE 2*a*) the disintegration of the outer root sheath in the region near and just above the bulb is nearly complete. The longitudinal palisade vessels are still intact, and the rich anastomoses of these vessels that

FIGURE 2. (*a*) A later stage of catagen. Contraction of the connective tissue sheath is more pronounced than in FIGURE 1*b*; the outer root sheath is more corrugated and capillaries are no longer discernible in the dermal papilla. Alkaline phosphatase. ×105. (*b*) Early catagen. A section cut tangential to the follicle with portions of the lower plexus and the outer root sheath showing. The black structure in the upper right is part of an eccrine sweat gland. Alkaline phosphatase. ×105.

form the lower plexus are as patent as in an anagen follicle, but the capillaries entering the dermal papilla no longer have crisp outlines. Instead the enzyme activity associated with them is diffuse, giving an over-all murky appearance to the papilla. This is perhaps an indication that the endothelial cells are breaking up and that the enzymatic activity that had been restricted to them has spread among the mesodermal cells. This degeneration of the capillary loops within the dermal papilla is the first indication of collapse in any of the follicular blood vessels. In the region of the bulb, the connective tissue sheath has undergone additional basal and central constriction. Its outline is irregular and it is definitely pinched in around the straight blood vessels supplying the dermal papilla. The degenerating hair bulb has also been pushed slightly up toward the skin surface.

In the succeeding phases of catagen the lower two thirds of the hair follicle are almost totally destroyed. During this process the blood vessels surrounding the connective tissue sheath show no evidence of degeneration and retain their sharp outlines. Indeed, the greatest amount of destruction of the follicle is in the region where the blood vessels are most abundant. Tangential sections through such catagen follicles show that the pattern of the vessels in the lower plexus frequently corresponds with the annular furrows that are present in the external root sheath. FIGURE 2*b* shows this relationship in an early catagen follicle, while FIGURE 3*a* illustrates the persistence of the plexus and its association with the corrugated external root sheath in an advanced catagen follicle. FIGURE 3*b* is similar to FIGURE 3*a* except that the plane of sectioning shows more of the lower plexus. The external root sheath is not visible in this preparation because it has not been counterstained.

In advanced catagen, most of the lower two thirds of the follicle is resorbed. The process of resorption is shown in FIGURE 4*a* and *b*. In FIGURE 4*a* the catabolic wave is approaching its upper limit near the attachment of the arrectores pilorum muscle. The connective tissue sheath is constricting distally and contracting around the entire lower portion of the follicle. The intricate network of blood vessels within the connective tissue sheath is still intact; the outline of the sheath has become slightly wrinkled during contraction. The glassy membrane is greatly thickened in its lower portion, but at the level where the club hair is forming it is much thinner. Numerous interdigitations and occasional spaces characterize the border between the glassy membrane and the external root sheath. The outer sheath has undergone extensive degeneration. In the lower part of FIGURE 4*b* the destruction is almost total and only a thin rim of the outer sheath remains. In the upper part of the figure, at the level where the final brush hair of telogen is forming, the external root sheath is still unscathed. Changes are also taking place in the internal root sheath. The cells in Huxley's layer are atrophic. The hair shaft itself is being resorbed. The buckling of the hair shown in FIGURE 4*a* is a result of the softening of the keratin and the constant upward force exerted on the shaft by the contraction of the connective tissue sheath. In FIGURE 3*b* the dissolution of the lower part of the hair is also evident. The lower bulb and the dermal papilla in follicles of advanced catagen (FIGURE 4*a*) are phosphatase-reactive and the deposition of the dark end product of the histochemical reaction obscures their structure.

The enzyme activity is not confined to the blood vessels but is diffuse; apparently an extension of the process noted in the earlier phase of catagen.

FIGURE 5*a* shows a hair follicle near the end of catagen. Brush formation is nearly complete and all of the hair shaft beneath the club has been resorbed. A thin cord of cells connects the dermal papilla and remnants of the hair bulb to the club hair. There are no capillaries in the dermal papilla and the lower plexus of the anagen follicle now forms a tight bundle of vessels around and

FIGURE 3. (*a*) Advanced catagen. The upper portion of a follicle where the club hair is forming at the level of the arrector muscle (arrow). The lower plexus is intact and the annular furrows in the outer root sheath are obvious. Alkaline phosphatase. ×105. (*b*) Advanced catagen. The lower portion of the hair shaft (arrow) is being resorbed. The lower plexus stems from the longitudinal palisade vessels. This section was not counterstained so the external root sheath is not readily apparent. Alkaline phosphatase. ×240.

beneath the papilla. This is a result of the progressive constriction of the connective tissue sheath.

At the termination of catagen, the dermal papilla has been pushed upward so as to lie just beneath the club hair, and a short stalk of cells, the papilla

FIGURE 4. (*a*) Advanced catagen. The connective tissue sheath is wrinkled and the softened hair shaft has buckled. Alkaline phosphatase. ×105. (*b*) Detail of *a*. Note the progressive wave of destruction ascending along the outer root sheath. Spaces may be seen (arrow) between the external sheath and the glassy membrane. ×220.

rest, connects the dermal papilla with the club. Some vessels of the lower plexus still persist in the connective tissue and form a bundle at the base of the follicle (FIGURE 5*b*). A hair follicle with these characteristics is said to be in the resting or quiescent state (telogen).

FIGURE 5. (*a*) Late catagen. Blood vessels in the connective tissue sheath are bunched below and around the avascular dermal papilla (arrow). A cord of cells connects the papilla with the club hair. Alkaline phosphatase. ×105. (*b*) Telogen. The connective tissue sheath surrounds the club hair and is constricted around and beneath the free dermal papilla. Remnants of the lower plexus are gathered below the papilla. Alkaline phosphatase. ×48.

Discussion

Catagen is not initiated by the collapse of the blood vessels within the dermal papilla. The destruction of the lower two thirds of the follicle is already under way before the capillary loops are affected. The erosion of the lower bulb, the appearance of annular furrows in the outer root sheath, the thickening of the glassy membrane and the constriction of the external root sheath are evident in early catagen when the blood vessels within the papilla are still patent. With the technique employed in this study, it is of course impossible to determine precisely when blood flow through these vessels ceases, but the contraction of the connective tissue sheath about the base of the follicle seems to clamp off the straight blood vessels entering the stalk of the dermal papilla, and the papillary loops become atrophic shortly thereafter. Thus the collapse of these blood vessels seems to be a result rather than a cause of catagen.

The plexus of blood vessels surrounding the lower part of the follicle remains intact through most of catagen. Some of them are still evident at telogen. This plexus is embedded in the connective tissue sheath, and invests the region where there is the most destruction of the follicle. In anagen hairs, the lower plexus has been associated with the very active outer root sheath[1] that contains abundant glycogen[6,8] phosphorylase,[8,10] esterases,[6,8,11] and beta-glucuronidase[8,12] and with the keratogenous zone, which is the first site of labeled cystine uptake in the skin.[13] The synthetic activities of anagen seem to be reversed in catagen. Glycogen is lost,[5] the external root sheath becomes atrophic, and the hair shaft is resorbed. Some cells in the dermal papilla are phagocytic,[14] but they probably play only a minor role in the destructive process of catagen. Since the lower plexus is patent throughout this period, these vessels are most probably the channels through which the catabolic products are passed off into the general circulation.

The activity of the connective tissue sheath during catagen may be visualized easily with the alkaline phosphatase technique, since the blood vessels embedded in it are strongly reactive for the enzyme. In the mouse the connective tissue sheath becomes greatly wrinkled during catagen;[5,9] in the human in wrinkling is less obvious. It does, however, undergo a progressive constriction that starts below the bulb during early anagen and moves upward like a peristaltic wave. The connective tissue sheath contracts around the base of the bulb, squeezing the degenerating bulb and the dermal papilla upward toward the telogen position.[14] This pressure is maintained during the entire process of catagen and is sufficient to buckle the softened hair shaft.

From the observations presented in this paper it seems likely that catagen may differ in humans and the mouse. Admittedly, the changes noted in the current report are rather gross. The cytology and histochemistry of the various structures that comprise the hair follicle have not been studied, and only thick sections processed for a single histochemical technique and counterstained with a nuclear dye were examined. These observations cannot, therefore, be compared to the exacting cytological studies of catagen in the mouse.[9] Aside from the differences already noted in the connective tissue sheath, there seem to be gross differences in the formation of the club hair. In man the

lower third of the hair shaft is resorbed, and a club hair is formed from the remnant at the level of the arrector muscle. In the mouse the club forms just above the hair bulb and is pushed upward to the telogen level. The blood vessels surrounding the hair follicles of man and the mouse also vary markedly. However, the vascular patterns around the anagen monotrich follicles in the rat[15] are quite similar to those associated with anagen hairs in the human scalp. Possibly catagen in the rat monotrichs might be more like that in man.

The origin of the glassy membrane has been debated extensively in the literature.[9] Some investigators believed it was a mesodermal derivative, some an ectodermal, and others thought it was derived, in part, from both. In human catagen a sharp line of cleavage separates the glassy membrane from the external root sheath. Frequently a space may separate the two layers. The glassy membrane, however, is always intimately associated with the connective tissue sheath. This is evidence of its pure mesodermal origin, and this is certainly in agreement without present knowledge of its homologue, the basement membrane.

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Part III. Alopecia Areata

THE CLINICAL ASPECTS OF ALOPECIA AREATA, TOTALIS, AND UNIVERSALIS

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The incidence of alopecia areata and totalis indicates a slight increase as determined by the number of patients treated in hospitals, in physicians' offices, and by the reports appearing in the medical literature.

Macalpine[1] calculates that the incidence of alopecia areata among 3,220 mentally defective patients was four tenths of 1 per cent; of the general popuation, she believes that 2 per cent of dermatological patients have the disease.

The age groups that are afflicted with alopecia areata are from childhood to the sixth and seventh decades of life. In a series of 68 patients treated in my office I observed that a larger percentage of males is affected. The diagnosis of alopecia areata is made by clinical appearance of the lesions that may occur on the scalp, face, extremities, and the body.

Examination of the involved alopecic areata usually demonstrates no evidence of macroscopic inflammation or irritation. The hair follicle is patent and the remaining hairs that are present, when removed, resemble exclamation points. The loss of hair may be preceded by the subjective complaint of pain in the scalp or headache. The falling of hair is usually rapid, and frequently the alopecia is noticed by another person.

The afflicted area at first is hyperemic, and then assumes a blanched hue. Sensation is normal at first, and then shows a slight hypoesthesia that is evidenced by the amount of stimulation with chemicals it can tolerate.

A differential diagnosis must be made by the clinician. Discoid lupus erythematosus, pseudopelade or alopecia cicatrisata, pattern alopecia, alopecia due to trichotillomania, lichen planopilaris, and tinea capitis must be ruled out before commencement of therapy.

The incidence of prepubertal alopecia is small. However, it is seen with enough frequency to be significant as an entity. In several of our preadolescent patients afflicted with alopecia areata there has been evidence of symptoms of Froelich syndrome.

The alopecia totalis is usually produced by a joining of several alopecia areas, which coalesce until the entire scalp is devoid of hair, producing the glistening scalp that becomes as predominant as a beacon light. Alopecia universalis, indicating loss of hair of the entire cutaneous surface, is usually the final stage of the alopecia case.

Attempts to correlate the frequency and the severity of mental disorders in patients with alopecia areata have been made by Greenberg,[2] who carried out a psychiatric survey of 48 adolescents and adults who sought dermatological aid for alopecia areata of the scalp. Greenberg found that 73 per cent of these cases were psychoneurotic, 9 per cent were borderline psychotic, and 6 per cent schizophrenic; 2 per cent had involutional psychosis. There was no conforma-

tion to any single personality pattern but the majority tended to be withdrawn and passive. Depression or anxiety were frequently prominent features. The implication of an acute emotional stress, as death in the family, witness to a fatal accident, or sudden economic reversal was extremely difficult to evaluate.

In the series of cases under my care and observation there was no significant evidence of psychopathic disorders in the patients. It is my belief that the psychoneurosis follows the onset of the alopecia which is fraught with emotional distress and insecurity. I suggest that it is illogical to conclude that patients with alopecia of many years' duration were originally pyschoneurotic, psychotic, or schizophrenic.

Specific etiological factors have not been definitely established. However, neurogenic, hormonal, and enzymatic relationships are significant.

In my practice, I have under observation three brothers and a mother, daughter, and son comprising two different families. Hereditary linkage may be a factor in these cases.

The diminished facial and scalp pigmentation of alopecia totalis patients, the regrowth of hair without pigment and, later, reappearance of facial and hair pigmentation in the successfully treated patient suggests a disturbance in pigment function. Van Scott discusses this phenomenon in greater detail elsewhere in this publication.

Kopf and Orentreich[3] have reported the diminution of alkaline phosphatase activity in the hair papilla during the early stages of alopecia areata. In the intermediate stage the alkaline phosphatase appearance has become intensely predominant.

Van Scott, elsewhere in these pages, has described the relation of the dermal papilla and hair bulb in the normal and the alopecic scalp. He states that in alopecia areata, both matrix and papilla are small but that the degree of the reduction of the matrix is greater than that of the papilla. In alopecia areata the regrowth of hair with corticosteroid therapy is associated with the enlargement of the matrix toward the normal size.

Laboratory studies that have included urinalysis, complete blood counts, protein bound iodine, sedimentation rate, and 17-ketosteroids have demonstrated no significant aberrations from the normal.

In many cases of alopecia areata of recent origin, remissions are observed with complete or partial regrowth of scalp hair. In patients who have had this type of alopecia for more than five years, however, it is rare for spontaneous regrowth to be observed.

In the alopecia universalis type, spontaneous regrowth of all the lost hair almost never occurs.

Dillaha and Rothman[4] postulated that hormonal factors may play a role in the initiation and clinical course of alopecia areata and totalis. They observed that during pregnancy patients with alopecia areata or totalis spontaneously recovered and then recommenced to lose their hair with the onset of menstruation. The original article described the use of the corticosteroids in the treatment of alopecia areata and totalis.

The metabolic effects of the corticosteroids—cortisone, hydrocortisone, prednisone, prednisolone, and methyl prednisolone—are made evident by the

ability of these steroids to initiate changes in sodium chloride and water retention, potassium diuresis, increased excretion of nitrogen and uric acid, and an increase in the blood and the urinary glucose level.

Burgoon *et al.*[5] have reported similar satisfactory results in patients affected with alopecia totalis.

Wilson[6] in England described the treatment of four patients afflicted with alopecia areata and universalis by the intravenous injection of corticotropin. In these cases of alopecia there was rapid regrowth of the hair of the scalp hairs, beard, and eyebrows. After the intravenous corticotropin therapy was discontinued, the loss of the new hair and the return of the alopecia totalis was observed 18 to 21 days after the commencement of therapy.

Huriez and Rony[7] injected a hydrocortisone acetate suspension subcutaneously into the scalps of patients with alopecia areata and totalis with subsequent regrowth of hair in the injected area. However, several weeks afterwards the new hairs fell out of their follicles.

In an earlier paper[8] I described the treatment of 42 cases of alopecia areata, totalis, and universalis with cortisone, hydrocortisone, prednisone, and prednisolone. These patients also received injections of 40 units of zinc ACTH gel weekly early in the treatment, and subsequently one intramuscular injection of 40 units of ACTH at monthly intervals. The corticotropin was used to prevent the suppression of the adrenal gland function during long-term corticosteroid therapy. However, there has been some evidence that the ACTH therapy may not be necessary in long-term corticosteroid therapy. Of the total number of 42 patients initially under treatment, complete regrowth was seen in 19 patient (45 per cent), and partial regrowth was observed in 23 (55 per cent).

When the scalp hair growth appears, it usually becomes visible as early as four weeks after oral corticosteroid treatment. In the majority of cases the new hair is usually white and devoid of pigment. However, as the hair develops in size and thickness, the original color of the normal scalp hair is seen. The percentage of undesirable side effects observed in the so-called Cushingoid syndrome, as moon facem hirsutism, acne, round back, and weight increase, was small. Hypertension, electrolyte imbalance, glycosuria, and gastric distress were notably reduced with oral prednisone and prednisolone. Gastric perforations or hemorrhages were not observed in any of the patients in this previous study.

During the past two and one half years, a clinical evaluation with methyl prednisolone (Medrol*) has been conducted to determine the effectiveness of this product in patients who have been afflicted with alopecia areata, totalis, and universalis. Two types of patients were treated: those who had been progressing favorably, and those who were not satisfactorily reacting to cortisone, hydrocortisone, and prednisolone therapy and had been placed on methyl prednisolone. The present series consisted of 68 cases of alopecia (TABLE 1). The duration of the alopecia ranged from 5 months to 25 years. The results of therapy indicated that 24 males had complete regrowth and 15 males had partial or poor regrowth; 17 females had complete regrowth and 12 females had

* Methyl prednisolone (Medrol) was furnished by The Upjohn Company, Kalamazoo, Mich.

partial or poor regrowth. In seven of the patients of the original series who were refractory to hydrocortisone or medicinal treatment and demonstrated sparse hair growth, the subsequent transfer to Medrol therapy was followed by an adequate and satisfactory hair growth.

The initial dosage in the majority of cases was 12 to 16 mg. daily, which was usually continued for 4 weeks. If there was no obvious hair growth the dosage was then increased to 20 mg. daily. When adequate hair growth was visible and maintained, there was a gradual lessening of the daily dose from 16 to 12 mg., and an attempt was made to establish a minimal corticosteroid dose at which hair growth was optimal. This maintenance dosage usually ranged from 8 to 12 mg. methyl prednisolone daily and a monthly injection of zinc corticotropin—40 units.

There was no specific pattern of hair growth; much variability was noticed in each patient. The chronological order of appearance of new hair was usually first on the scalp, face, eyebrows, forearms, arms, body, and pubic area and,

Table 1

Chemical Aspects of Alopecia Areata, Totalis, and Universalis

Diagnosis	No. of patients	Good growth	Partial growth	Poor growth
Alopecia areata	23 males	16 or 70%	7 or 30%	
	10 females	6 or 60%	4 or 40%	
Alopecia totalis	8 males	3 or 37%	5 or 63%	
	15 females	8 or 53%	7 or 47%	
Alopecia universalis	8 males	5 or 63%	2 or 25%	1 or 12%
	4 females	3 or 75%	0	1 or 25%
Totals	68 patients	41 complete (60%)	25 partial (37%)	2 poor (3%)

Reproduced by permission from *Medical Times*.[8]

usually, the eyelashes last. The growth of the eyelashes and eyebrows was always nonpredictable, particularly as to the rate of growth and permanence. Frequently I noticed that when the regrowth of the scalp hair became demonstrable and heavy, the new hairs of the eyebrows and eyelashes were lost or maintained in a bizarre fashion.

When there was no evidence of adequate hair growth after 4 to 6 months of continuous corticosteroid and corticotropin therapy, I considered these cases as treatment failures, and the withdrawal of the steroid therapy subsequently was progressively reduced in an attempt to prevent the appearance of unfavorable symptoms, as muscle fatigue or pain, fever, lassitude, and restlessness. Corticotropin was then given.

When a patient is selected for long-term corticosteroid therapy for the treatment of refractory alopecia areata and alopecia totalis, the treatment and its occasional consequences must be explained to the patient in order to obtain his fullest cooperation. The patient is advised to contact the office if there is noted severe epigastric pain, blood in the stool, excessive muscular weakness, or intercurrent infection, particularly respiratory. Blood pressure readings and urinalyses were performed regularly in order to ascertain any early indi-

cation of untoward effects as a result of the prolonged administration of corticosteroids. Patients were advised to maintain a restricted salt diet. It was not considered necessary to prescribe potassium salts in order to prevent a possible hypopotassemia in these patients. If there was excessive retention of body fluid then patients were advised to restrict food intake. Dextroamphetamine was prescribed: 10 mg. daily, orally, as an appetite depressant. Occasionally chlorothiazide was utilized orally to aid in diuresis and subsequent reduction of fluid.

The emotional and physical discomfort experienced by alopecia patients can be compared to the physical discomfort observed in the rheumatoid diseases and severe asthma.

In commencing treatment of alopecia areata, totalis, or universalis with corticosteroid and corticotropin, an intelligent and cautious selection of patients must be made. It is extremely gratifying to the patient and the physician to observe the progressive regrowth of hair, and the return of a normal and calm mental outlook of a previously emotionally disturbed patient. I believe that is is essential to select a corticosteroid that is the most effective and the least toxic (therapeutic index) in the therapeutic armamentarium today.

These patients who are on long-term corticosteroid therapy must be observed clinically at regular intervals, and periodic laboratory determinations must be conducted of the urine and of the blood for glucose and electrolyte disturbances. Also, when necessary, diagnostic X rays must be taken to rule out depletion of bone calcification and osteoporosis. The modus operandi of the effectiveness of corticosteroid and corticotropin therapy is conjectural. The removal of a hair growth inhibition factor, as previously suggested by Chase[9] and Mercer, must be considered. If the alopecia areata entity is due to an inflammatory or allergic component, as suggested by Van Scott, then the antiphlogistic and antiallergic effects of the steroids must be advanced.

The results of the above studies indicate that in a significant percentage of alopecia areata, totalis, and universalis patients, hair growth can be stimulated by adrenocorticotropin and corticosteroids. However, the nonpredictability of these findings must serve as a stimulus for continued intensive research in an attempt to correlate etiology, pathogenesis, and corticosteroid effect.

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AUTOGRAFTS IN ALOPECIAS AND OTHER SELECTED DERMATOLOGICAL CONDITIONS

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Skin autografts have been employed in animals to study hair growth,[2-4, 7-9] pigment formation,[1-2,5-7] wound healing,[10-11] and immunity.[12] Exchange autografts were performed in man to study vitiligo,[13-15] amyloidosis,[16] morphea,[15] scleroderma,[13] acrodermatitis chronica atrophicans,[15] allergic eczematous dermatitis,[17] fixed drug eruptions,[18-22] and hyperidrosis.[23] The effects of autografts have been observed following plastic repairs for lupus erythematosus.[24]

Autografts to the eyebrow, hand, scalp, and other areas at times have shown not only the hair growth desired on such grafts but also, occasionally, the development of unwanted hair.[25-41]

Method

Multiple transposition of skin punch free grafts was performed in order to study some factors in the pathogenesis of certain dermatological disorders, especially the alopecias. After local anesthesia, and appropriate surgical preparation of the skin, which included washing, shaving, and cleansing with alcohol, 4 full thickness circular excisions were made with punches of 6, 8, and 12 mm. diameter (FIGURE 1). Two of the circular grafts were excised from a site of persistent disease, represented by the circle, and two circular specimens were excised from a normal site (FIGURE 2).

The grafts were removed, making certain that the excision was carried below the hair follicles. Each graft was trimmed of excess fat, and of the galea aponeurotica if present. The grafts were then transplanted in the following manner: (1) a normal graft was transplanted to a normal site; (2) a normal graft was transplanted to an affected site; (3) an affected graft was transplanted to a normal site; and (4) an affected graft was transplanted to an affected site.

Wherever feasible the grafts were transposed in clockwise fashion (FIGURE 3*c*).

Care was taken to set the grafts so that the possible growth of the hair would be in the desirable direction (FIGURE 3*b*). Hemostasis was obtained by direct pressure for 20 to 30 minutes.

Fixation of the graft was accomplished by fibrin clot, Telfa (perforated plastic dressing), Scotch tape, adhesive tape, collodion (FIGURE 4*a*) or sutures (FIGURE 3*a*, *b*, and *c*). The 6- and 8-mm. grafts rarely required sutures. The 12-mm. grafts were all sutured in place. The sutures were inserted near the graft and carried over the graft. No suturing was done into or under the graft. Sutures and other dressings were removed after the sixth to ninth day (FIGURE 3*d*). Photographs were taken before and after the procedures, and at about monthly intervals thereafter.

The experiments were performed in studying the following maladies affecting hair growth:

Disorder	*Number*
Alopecia prematura	52
Alopecia areata	9
Alopecia cicatrisata	3
Woolly hair nevus	1

FIGURE 1. Punches of various diameters.

Alopecia prematura. The 52 subjects with alopecia prematura (common male pattern baldness) were white men, ages 19 to 50 years. The longest follow-up period in the study was 2½ years (FIGURE 7*b*). In some of these patients grafts were repeated on several occasions.

Alopecia areata. There were 4 women and 5 men, including one Negro, with alopecia areata. Their ages varied from 22 to 42 years. The follow-up period was 1 to 1½ years. In one of the patients the experiment was repeated.

Alopecia cicatrisata. Three white women, aged 50, 54, and 58, had alopecia cicatrisata. Clinically and, according to the history, they were probably "burned out" cases of pseudopelade of Brocq. The follow-up period in these patients was 1½ years.

Woolly hair nevus. One Negro woman, age 37, with woolly hair nevus, was observed for one year.

Psoriasis and vitiligo. Two female patients with vitiligo and one with psoriasis were observed for more than one year. One of the patients with vitiligo had the grafting procedures performed twice, in different sites of course.

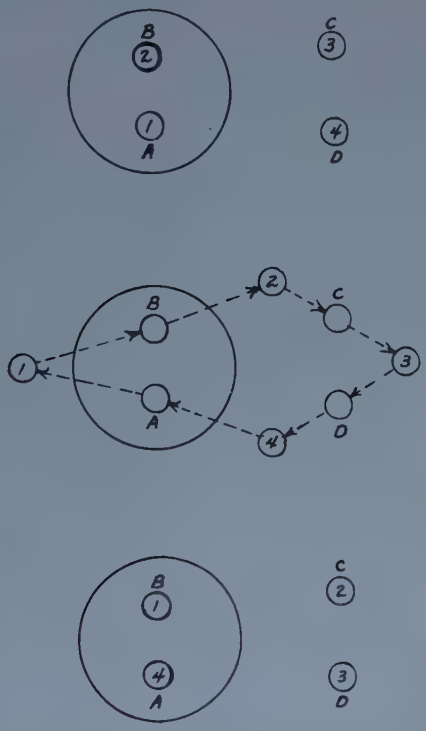

FIGURE 2. The large circle represents the site of persistent disease. Punch grafts are taken at A, B, C, and D. Grafts 1, 2, 3, and 4 are transplanted: 1 to B, 2 to C, 3 to D, and 4 to A.

Results

Of a total of 284 punch grafts, only one of the punch grafts failed to take. This was in a patient with alopecia areata in whom a graft with hair fell out from the recipient alopecia site. This graft had been covered with collodion. Its loss was noticed the next day and it was replaced by a new hair graft that took without difficulty.

Generally the grafts developed a superficial crust that fell off in 1 to 2 weeks (FIGURE 7*a*, arrows 1 and 7). In 2 to 3 weeks and without exception the hairs were shed from hair-bearing grafts. FIGURE 4 shows the hair-bearing graft,

shaven of visible hairs, in its recipient site covered with collodion. FIGURE 4*b* shows these hairs 2 weeks later. Occasionally all the hairs are shed with the crust. Many of these hairs were examined microscopically. FIGURE 5*a*, *b*, and *c* show microphotographs of such hairs shed in patients with alopecia areata. They all show the features of telogen. Many had the shape that might be described as a "shepherd's crook" (FIGURE 5*a* and *b*).

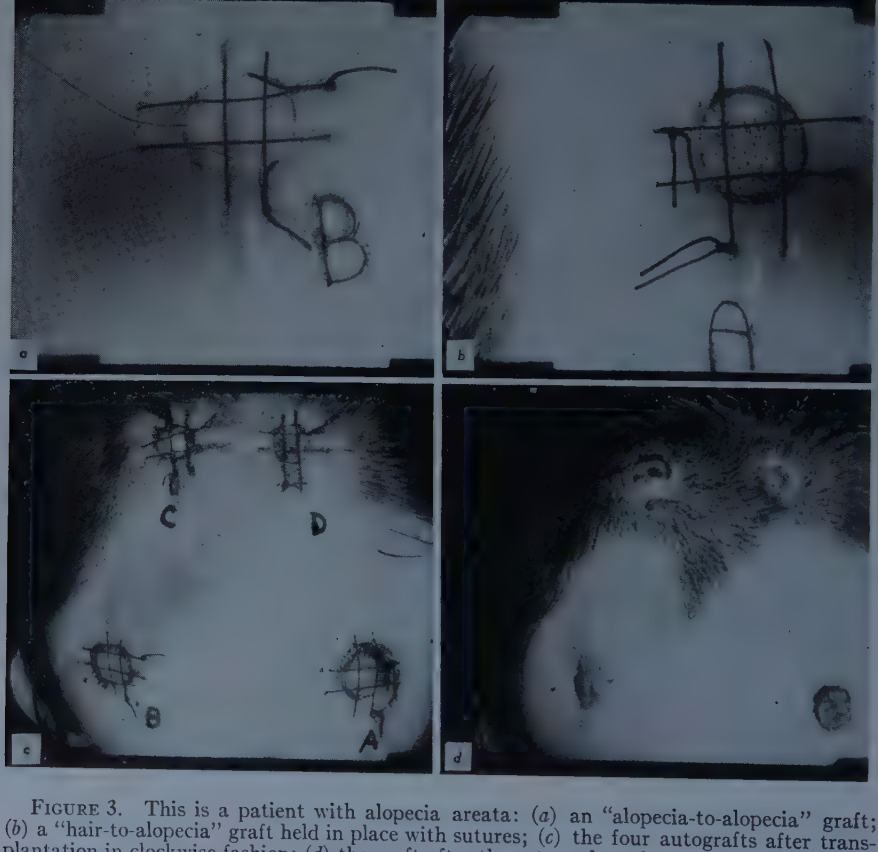

FIGURE 3. This is a patient with alopecia areata: (*a*) an "alopecia-to-alopecia" graft; (*b*) a "hair-to-alopecia" graft held in place with sutures; (*c*) the four autografts after transplantation in clockwise fashion; (*d*) the graft after the sutures have been removed.

In grafts in which hair regrowth occurred, the hair appeared above the surface two to three months after the procedure. FIGURE 7*a* shows grafts in different stages of hair growth. In a few months the line of union between the donor graft and the recipient site became almost invisible.

I suggest that the terms donor dominant and recipient dominant (FIGURE 6) be used to describe the following conditions: when the transposed grafted skin maintains its integrity and characteristics independent of the recipient site it is donor dominant; when the transposed grafted skin takes on the characteristics of the recipient site it is recipient dominant.

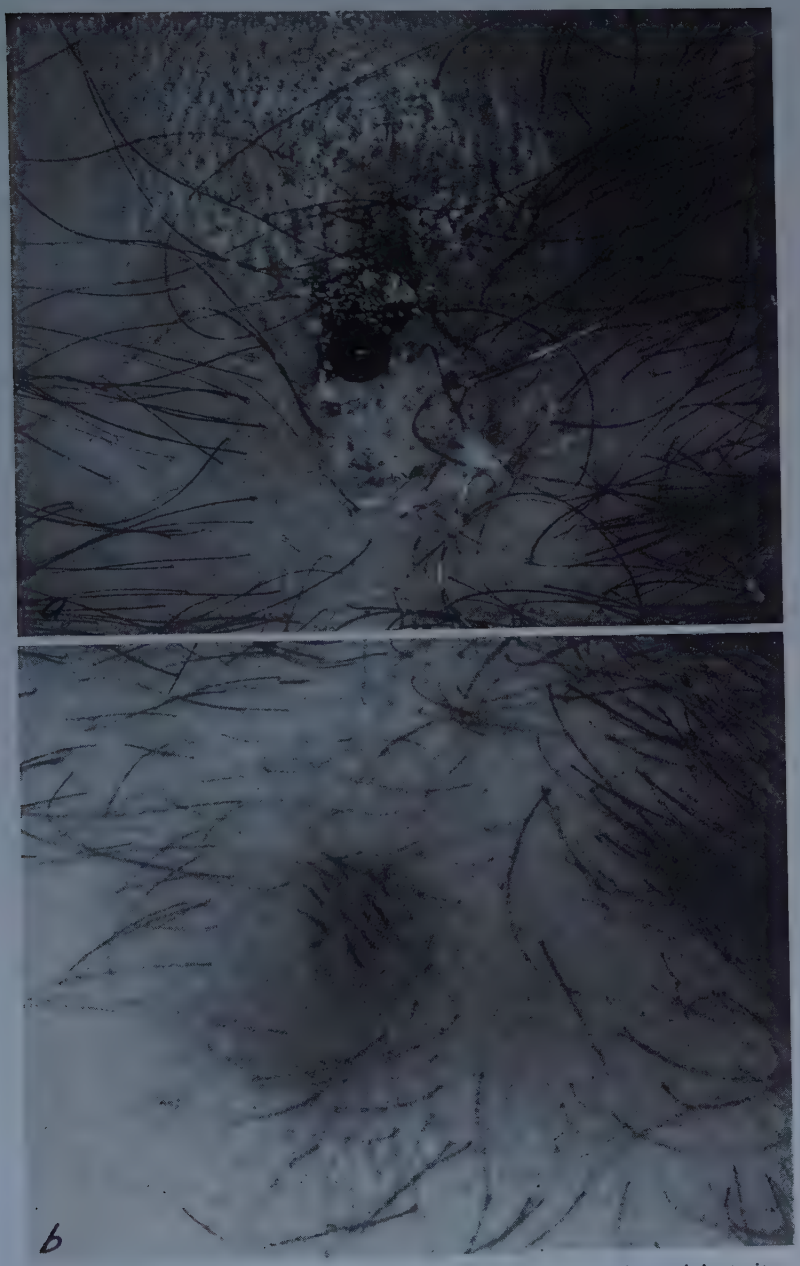

FIGURE 4. (*a*) The hair-bearing graft shaven of visible hairs in its recipient site. It is covered with collodion; (*b*) the hairs being shed two weeks later.

FIGURE 5*a*, *b*, and *c*. These microphotographs show types of hair shed from the grafts; (*a* and *b*) hairs of the "shepherd's-crook" type. ×213.

Alopecia prematura. Donor dominance was observed in all of the cases of alopecia prematura. "Hair to hair" grew hair; "hair to bald" grew hair; "bald to bald" remained bald; and "bald to hair" remained bald.

The grafts containing hair continued to grow hair in the area of alopecia that was of the same texture and color and, apparently, at the same rate and

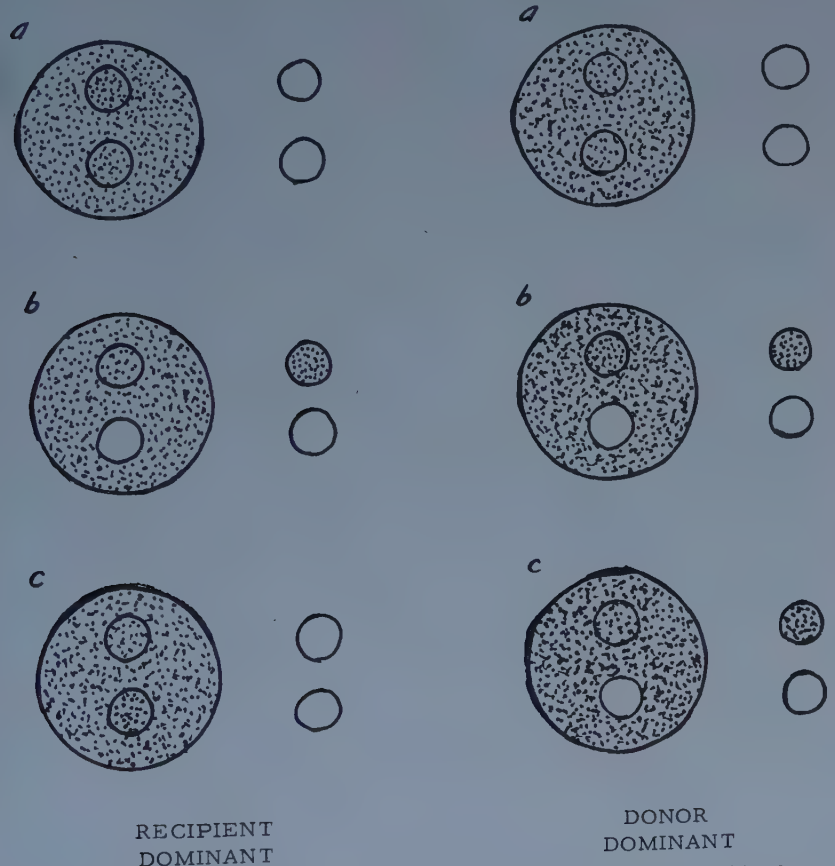

FIGURE 6. The stippled areas represent the sites of persistent disease: (*a*) where the punches were made; (*b*) the grafts after they were transplanted in a clockwise fashion; (*c*) the fate of the grafts after a period of time.

with the same period of anagen that governed the nature of the hair of the donor site (after 2½ years' follow-up it was still growing [FIGURE 7*b* and 7*d*]).

In several patients, grafts growing hair were implanted at the edge of a receding hairline. In the two years of observation following the grafting the hairline continued to recede at its preordained pace. The grafts, however, continued to show hair growth, greater and greater distances being manifest between the hair of the graft and continued recession of the hairline. Moreover, the hair growth of the grafts appeared unimpaired.

The subject presented in FIGURE 7*b*, *c*, and *d* shows the growth of hair in

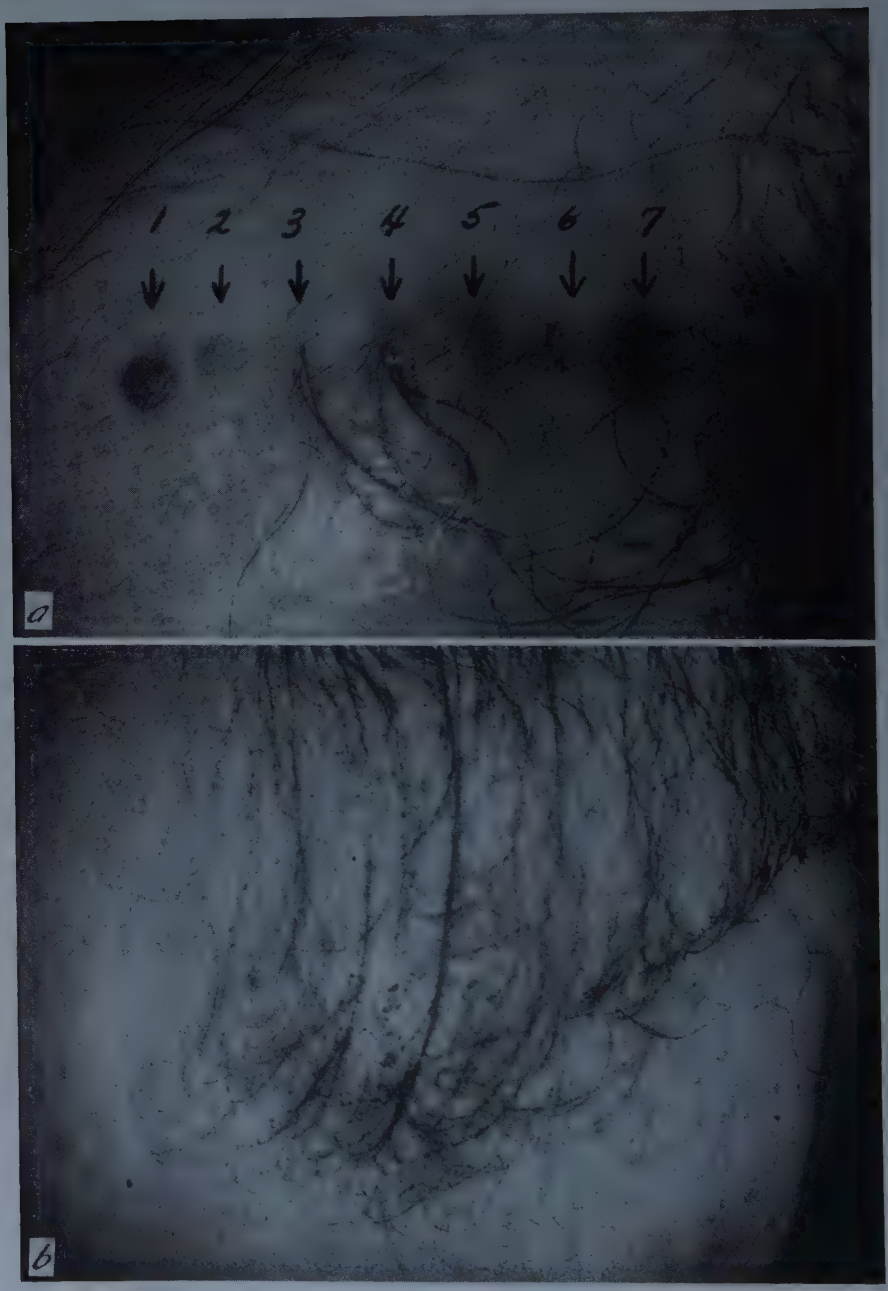

FIGURE 7. A patient with alopecia prematura: (*a*) grafts at various stages following transplantation. Grafts 1 and 7 show crusts one week after grafting. Grafts 2, 5, and 6 show healing one month later. Grafts 3 and 4 show hair growth 6 months later; (*b*) hair growing on the patient's left frontal scalp.

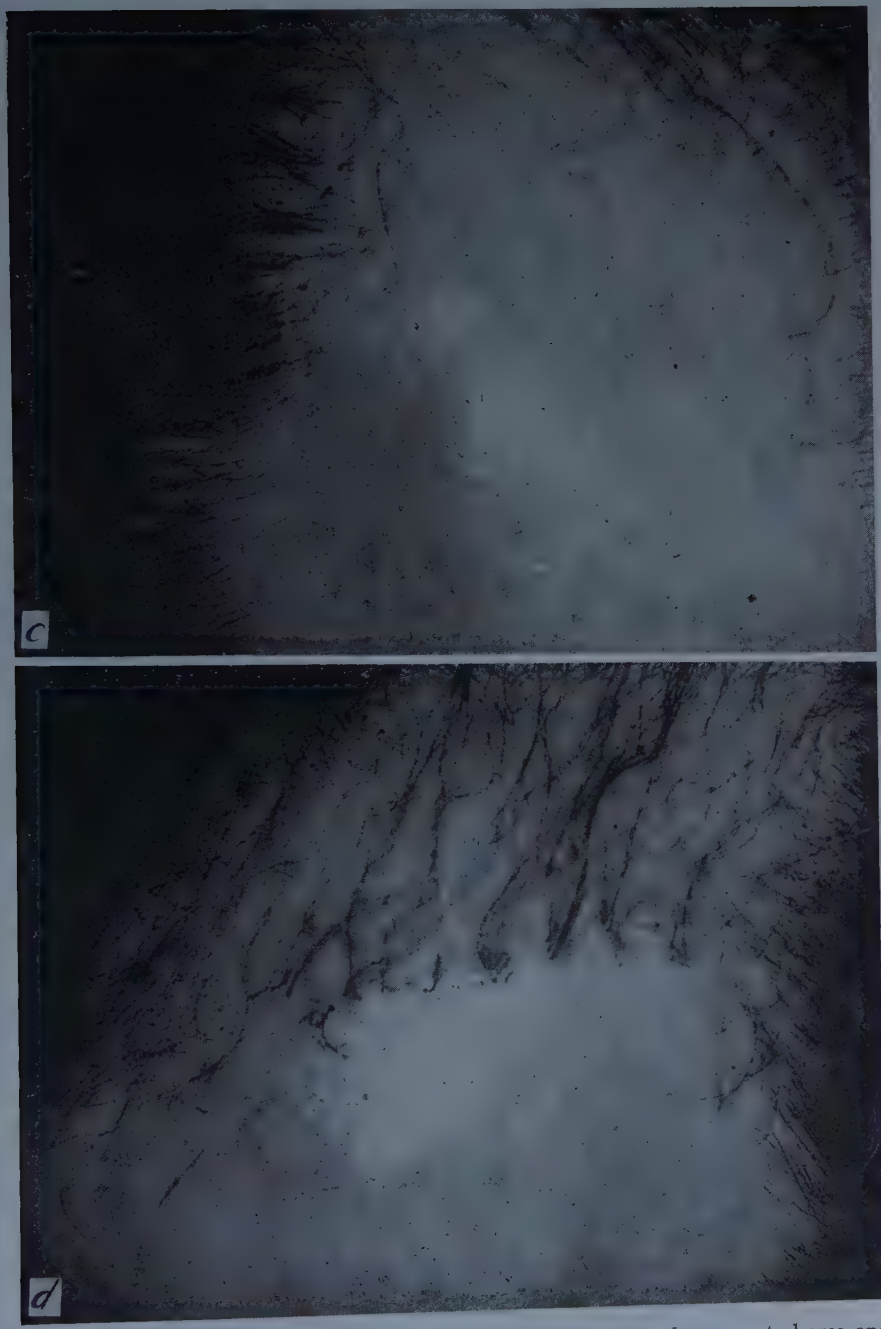

FIGURE 7*c*. A close-up of the right frontal scalp. This was used as a control area and stayed bald; (*d*) a close-up of the left frontal scalp area, where the grafts have continued to grow strong scalp hairs for the past 2½ years.

the graft 2½ years after its transposition to the left frontal scalp. The right frontal scalp lacks hair, this area having been used as a control.

Alopecia areata. Attempts to choose persistent and circumscribed sites of alopecia areata occasionally failed when there was rapid extension of the disorder to involve all 4 graft sites (FIGURE 9). Occasionally spontaneous hair regrowth would occur and thereby disturb the interpretation of the results.

In 5 of the 9 cases donor dominance was partial. However, in alopecia areata, unlike alopecia prematura, only sparse and weak hair growth took place in both the "hair-to-hair" grafts and the "hair-to-alopecia" grafts. The 'alopecia-to-alopecia" and "alopecia-to-hair" grafts did not grow hair.

FIGURE 8. A patient with alopecia prematura. The arrow indicates the site of a graft taken from a hair growing area in the posterior scalp. Two years ago, this had been implanted at the edge of the receding hairline (dotted line). Note receded hairline. Graft continues to grow hair.

Alopecia cicatrisata. Two cases of alopecia cicatrisata showed a relative "donor dominance," the hair growth, however, was very sparse. Only 2 velluslike hairs, 1 cm. in length, regrew from the 15 terminal hair follicles of the transplant. These have persisted for more than 1 year. One patient, however, had excellent terminal hair growth, indicating donor dominance (FIGURE 10).

Woolly hair nevus. The patient with the woolly hair nevus developed a keloidlike reaction in all the grafts and, of course, no hairs grew. The result was therefore inconclusive in regard to growth of hair.

Psoriasis. The patient with psoriasis (FIGURE 11) developed an isomorphic response (Koebner phenomenon). All 4 grafts became psoriatic.

Vitiligo. The multiple transposition experiment was performed in two white women with persistent vitiligo. They were followed for more than one year. "Recipient dominance" was observed in all sites in both patients.

Pigmented skin to pigmented skin remained pigmented. Pigmented skin to vitiligo skin became vitiliginous. Vitiligo skin to vitiligo skin remained vitiliginous. Vitiligo skin to normally pigmented skin became pigmented (FIGURE 12*a*, *b*, and *c*).

FIGURE 9. A patient with alopecia areata. There was rapid extension of the disorder to involve all 4 graft sites.

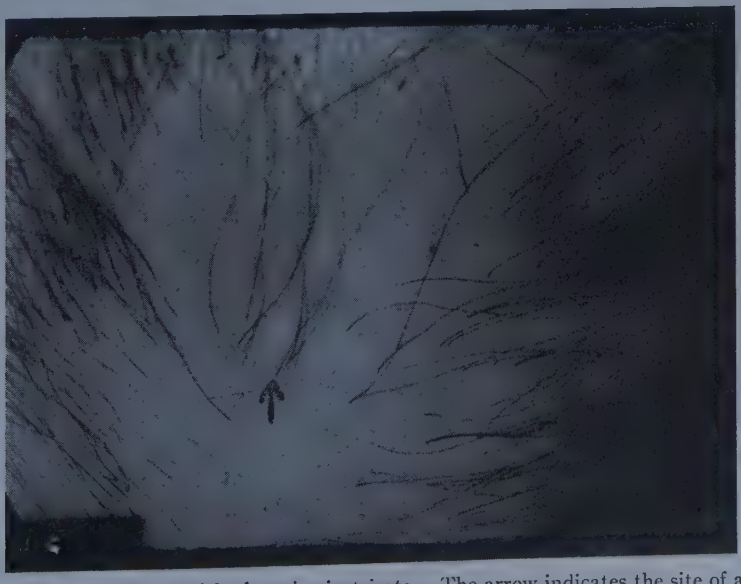

FIGURE 10. A patient with alopecia cicatrisata. The arrow indicates the site of a "hair-to-alopecia" graft performed 1 year ago. Terminal hair growth is shown.

Discussion

The use of autografts as a research tool has helped our understanding of many physiological and pathological phenomena of the skin. Previous studies have occasionally failed or have produced contradictory results. Moreover, confusion had resulted from transplants that had failed to take or had been only partially successful. Therefore the multiple controls of the present technique were instituted.

To achieve a successful transplant, sufficient nourishment (vascular recipient bed), primary tissue contact (pressure with postoperative immobilization),

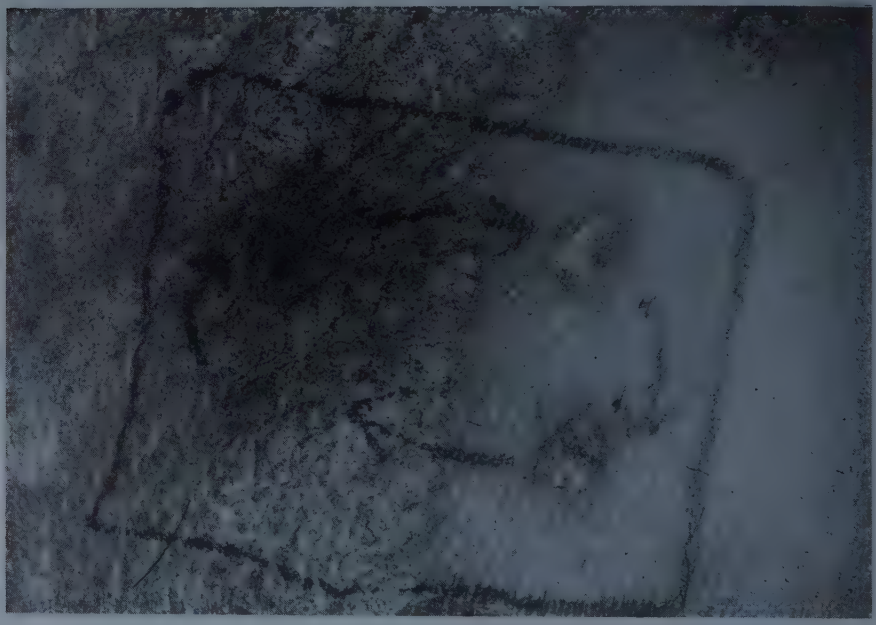

FIGURE 11. This patient with psoriasis shows the isomorphic phenomenon of Koebner following grafting.

asepsis, and control of excess bleeding are necessary. The first nourishment of the graft is plasma. There is an early anastomosis of small capillaries, and then new capillaries proliferate into the older vessels. Vascularization of a graft usually occurs in 3 days,[42] connective tissue attachment in 2 weeks, and fat layer appearance in 3 weeks. Sensation (pain, temperature, tactile) develops in a variable period of time (months to a year) usually starting at the periphery and proceeding toward the center of the graft. The 6- and 8-mm. grafts develop sensation within 2 months. It would be interesting to observe if in the larger grafts (12-mm. and more) significant difference in hair growth and sensations as between the periphery and the center become noticeable. This work is now in progress.

For alopecia prematura these studies would seem to indicate that the determinants of growth of strong scalp hair or of baldness lie within the local skin

FIGURE 12. A patient with vitiligo: (*a*) the crusts one week after grafting; (*b*) complete healing one month later; (*c*) "recipient dominance." The upper arrows indicate the site of the first series of grafts. The 4 sites below the arrows are a second series of grafts that had been transplanted in clockwise fashion.

tissues of a full-thickness graft and suggest that the pathogenesis of common male baldness is inherent in each individual hair follicle. Probably each individual follicle is genetically predisposed to respond or not to respond to androgenic and/or other influences that inhibit its growth. This would explain the frequent clinical finding of isolated normal-growing terminal hairs in areas of male pattern baldness. The results of the present experiment certainly pin point and agree with the statement that "the capacity for development of baldness appears to be controlled by factors, resident in localized areas of the scalp."[43]

This study would seem to refute theories of the pathogenesis of ordinary human baldness based solely on the "chronic activity of the scalp muscles (via branches of the facial nerves) that lead to shearing stresses in the dermis of the scalp and consequent ischemia."[44]

Although only 2½ years have passed since the performance of these autografts, there is evidence that local factors outside the hair follicular apparatus are not significantly related to alopecia prematura (male pattern baldness). In cases of receding hairlines in which hair-growing grafts were implanted at the periphery of the hair line, these grafts continued to grow normal hair, separated by 1 to 2 cm. from the front of the continually receding hairline. It is traditionally accepted that pressure and avascularity may result in alopecia. Clinically this is best seen over some sebaceous and epidermoid cysts of the scalp.

Although donor dominance was noted in alopecia areata, it was only partial. Even the hair-to-hair grafts failed to grow normal hair in any of the cases. It is possible, therefore, that trauma alone may precipitate some of the lesions or some of the variants of alopecia areata. In all grafts bearing scalp hair the hairs are always shed as a result of free grafting, and then anagen begins. It is stated that "in alopecia areata the hair bulb appears to be restrained from proceeding beyond the comparable stage of anagen IV (in the mouse) and fails to produce morphologically normal hair."[45] This may explain the failure of our hair-to-hair graft to grow more than weak, sparse hairs, since the hairs are now all in relatively early anagen. In order to arrive at more definite conclusions concerning alopecia areata, larger grafts are now being employed.

The results in alopecia cicatrisata indicated donor dominance. The poor growth of hair-to-alopecia grafts in alopecia cicatrisata may be explained by the poor vascularity of the recipient site.

The experiment in woolly hair nevus of a Negro woman failed because of the hypertrophic keloidlike reaction produced by the procedure.

The shepherd's crook-shaped hair that is shed from grafts with hair require biopsies to explain their formation. One may speculate that the hair papilla apparently rises into the dermis from the subcutis and carries the hair bulb with it faster than the hair shaft is released from its follicle, thus bending back on itself.

The case of psoriasis developed an isomorphic response (Koebner phenomenon). This may possibly be circumvented in the future by the use of large enough grafts that may leave the center free of this reaction to physical trauma.

Patients with vitiligo, unlike those with alopecia, show recipient dominance. Apparently systemic factors and/or deeper seated structures and tissues

determine the local reactions. It is possible that the nerves, blood vessels, and/or lymphatics govern the ulterior factors that influence skin changes.

This parallels the results and theories of others[12-24] in certain other dermatological lesions, such as those of fixed drug eruption, allergic eczematous contact dermatitis, and chronic discoid lupus erythematosus.

It is clear that possibly there may be far reaching significances of the techniques used here for elucidating the pathogenesis of skin disorders and for understanding the factors determining their localization.

Table 1 shows recipient or donor dominance of dermatological conditions after utilizing autografts. The dominance probably differentiates between those conditions in which the factors responsible for the production of the skin lesion reside in the skin itself and those in which the deeper tissues determine the localized reactions in the skin.

Table 1
Characteristic Autograft Dominance in Dermatological Conditions Studied to Date

Alopecia prematura	Donor
Alopecia areata	Donor
Alopecia cicatrisata	Donor
Hair growth cycle	Donor
Localized amyloidosis	Donor
Vitiligo	Recipient
Allergic eczematous dermatitis	Recipient
Fixed drug eruption	Recipient
Lupus erythematosus	Recipient
Morphea	Recipient
Acrodermatitis atrophicans	Recipient
Psoriasis	Isomorphic response

Summary

The technique of multiple transposition of full thickness autografts is described.

Sixty-eight patients were studied, including 52 with alopecia prematura, 9 with alopecia areata, 3 with alopecia cicatrisata, 1 with woolly hair nevus, 1 with psoriasis, and 2 with vitiligo.

The term donor dominance is introduced for skin autografts which maintain their integrity and characteristics after transplantation.

The term recipient dominance is introduced for skin autografts that develop the characteristics of the recipient sites.

In alopecia prematura donor dominance occurred.

In alopecia areata partial donor dominance occurred, but hair regrowth in all the grafts was stunted.

In alopecia cicatrisata a form of donor dominance occurred. However some interference of hair growth due to the scarring was observed.

In the patient with woolly hair nevus a keloidlike reaction prevented all hair growth.

In vitiligo recipient dominance occurred.

In psoriasis an isomorphic phenomenon was observed in all the grafts.

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EVALUATION OF DISTURBED HAIR GROWTH IN ALOPECIA AREATA AND OTHER ALOPECIAS

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Many specific chemical and physical agents, as well as disease conditions, are known to interfere with the growth of hair in man. With several of these the pathological changes in the scalp and the pathogenesis of the disturbed function of the hair follicle and hair root have been sufficiently studied to permit identification of the defect in each case and to allow classification of hair loss into several general types. In no instance, however, have specific biochemical or biophysical mechanisms of interference been fully established.

The etiology of alopecia areata is unknown even though speculations on causes of the disease may be somewhat numerous. The essential nature of the defect in hairs and hair follicles was recorded thirty years ago by Sabouraud,[1] who made the significant observation that hair follicles in this disease continually, although abortively, attempt to produce hair. The pathological state of the hair follicle in relation to its environmental connective tissue has been studied recently[2,3] in an attempt to understand more fully the nature of the defect in hair growth in alopecic states.

This paper endeavors to evaluate and interpret the problem of alopecia areata by comparing findings in this disease, of unknown cause, with findings in alopecic states where the etiology is known.

General clinical considerations of alopecia areata. Baldness in this disease is generally first noticed on the scalp as small circular areas, which remain circular in configuration as they enlarge. Similar areas of baldness simultaneously occur on other areas of the body but often are unnoticed at the onset of the disease. Baldness of the entire body surface may occur, and develops generally by coalescence of ever-enlarging balding patches, although the progress of the disease may be so rapid that loss of hair seems to occur on all parts of the body almost simultaneously. The configuration and variable size of the bald areas is not correlated with known anatomical divisions of the skin (for example, dermatomes, nerve supply, vascular supply, body region).

The course of the disease is unpredictable. There may be persistent localized areas of baldness, or large segments of the body may become bald, or hair may regrow in all areas, after which loss of hair may or may not recur again at a later time.

No objective systemic abnormalities indicative of a general toxicity, such as infection, fever, allergy, depressed formation of cells by the bone marrow, or abnormal endocrine states are known to be associated with the disease.

The cutaneous lesion of alopecia areata. Detectable abnormalities of the scalp seem to make their appearance abruptly, inasmuch as clinically uninvolved scalp adjacent to enlarging bald areas is found to be histologically normal. I have observed such a histologically normal scalp to become totally alopecic within a two to three week period following biopsy. The rapid development of a lesion thus makes it difficult to determine the sequential

development of histopathological changes that are found in lesions. The description of the lesion that follows, therefore, does not necessarily indicate the temporal order in which changes occur, but presents these changes in anatomical order from the deeper corium to successively higher levels of the corium.

In the lower levels of the dermis degenerativelike changes are seen in the connective tissue that surrounds the blood vessels leading to hair papillae (FIGURE 1*a*). A cellular infiltrate, not present at this lower level, appears perivascularly at a somewhat higher level (FIGURE 1*b*) and is particularly dense immediately subjacent to the dermal hair papilla (FIGURE 1*c*). This infiltrate discretely does not invade the dermal hair papilla (FIGURE 1*d*), but instead extends upward and laterally to form a zone of infiltrate that concentrically surrounds the entire hair bulb (FIGURE 2*a*). Above the hair bulb, the external sheath of the hair root may (FIGURE 2*b*) or may not (FIGURE 2*c*) be invaded by the inflammatory cells. A cellular infiltrate is minimal or absent in the upper half of the corium, that is, at the level of the sebaceous glands and above.

Certain abnormalities in the dermal hair papilla perhaps should be mentioned at this point. One is the not infrequent presence of cells in mitotic division (FIGURE 2*d*). Although cells in mitosis may occasionally be found in hair papillae normally, the frequency of their occurrence in alopecia areata seems to be greater than normal.

In the lesion of alopecia areata large amounts of melanin are found in the dermal papillae and underlying corium of hair roots in anagen phase of the growth cycle. Melanin granules may be found normally in papillae of hair roots in telogen phase of the growth cycle but are not found in papillae of normal anagen roots.

Although hair is clinically absent in lesions of alopecia areata, most hair roots within the bald scalp histologically are found to be in a proliferative state of activity and capable of producing an internal root sheath that appears to be normal in appearance and in size.[2] The shaft of hair produced, however, is diminutive in caliber and is incompletely keratinized.

The state of most hair roots in alopecia areata, in regard to hair production, is similar to the transient stage of early anagen in the mouse known as Anagen IV. In this stage, described and classified by Chase *et al.*,[4] the hair matrix produces a well-formed internal root sheath but forms a hair that is diminutive in diameter.

In light of the essential dependency of the growth of the hair upon an intact dermal hair papilla,[5-9] abnormalities in geometric relationships of the hair matrix and papilla that have been found in alopecia areata[3] are perhaps significant. In the normal scalp the volume of the matrix is approximately ten times the volume of the papilla. In alopecia areata the volumes of both are considerably diminished. However, the degree of reduction of volume of the matrix is disproportionately greater than that of the papilla, since the volume of the matrix is found to be only approximately three times that of the papilla. However, the number of mitoses found in the matrix per unit volume of matrix is normal, or even increased,[3] although of course the total number of mitoses in the smaller matrix is less than in the larger normal matrix. When hair

grows in response to prednisone therapy the matrix of the hair root tends to return toward normal in regard to the above parameters. On the other hand the hair papilla, at least within the first few weeks of prednisone therapy, shows few or no significant changes. The degenerative appearance of the

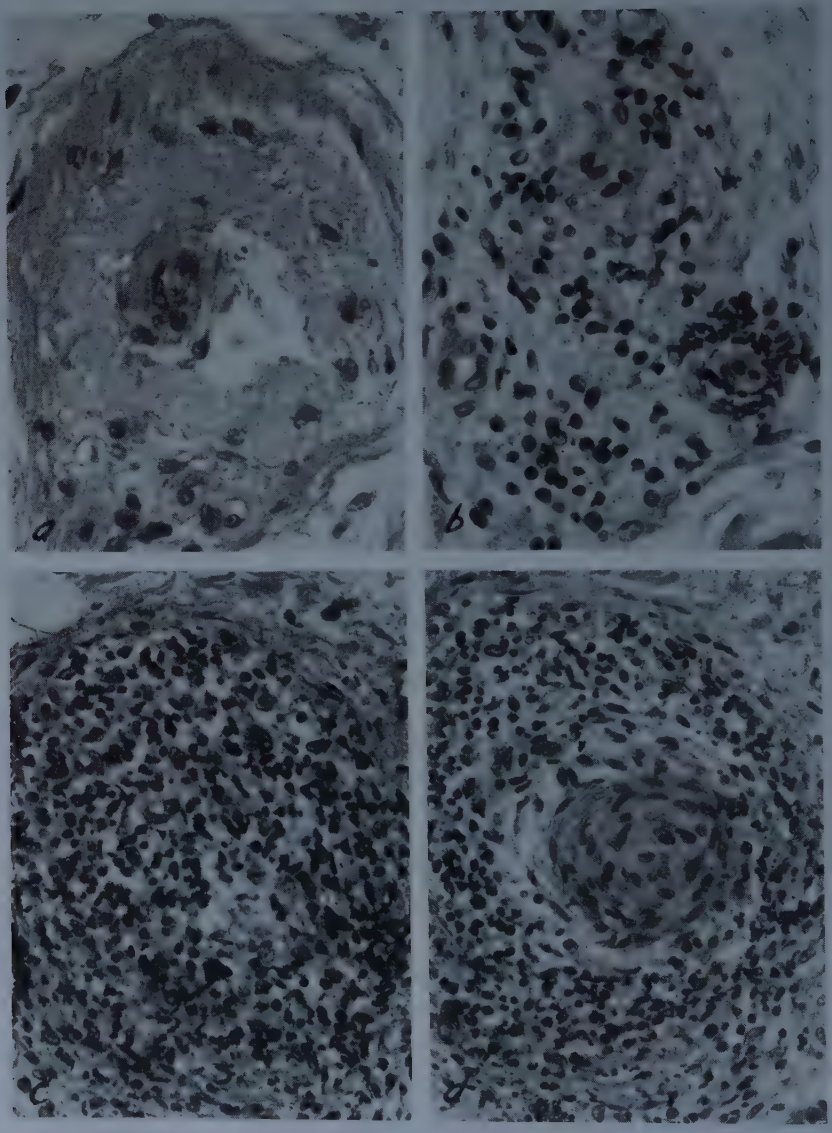

FIGURE 1. (*a*) Degenerativelike change in the connective tissue surrounding blood vessels supplying hair papilla. Horizontal section of scalp, deep corium. Gomori trichrome stain. ×465. (*b*) Moderate cellular infiltrate surrounding blood vessels supplying dermal hair papilla. Level of section about 60 μ above that of *a*. ×385. (*c*) Dense cellular infiltrate immediately subjacent to hair papilla. ×310. (*d*) Cellular infiltrate surrounding, but not invading, hair papilla. ×285.

connective tissue surrounding the subpapillary blood vessels, as well as the peribulbar cellular infiltrate, show little if any change during the same interval.

Appearance of epilated hairs in alopecia areata. Morphologic defects in epilated hairs provide a means for comparing alopecias of different types and,

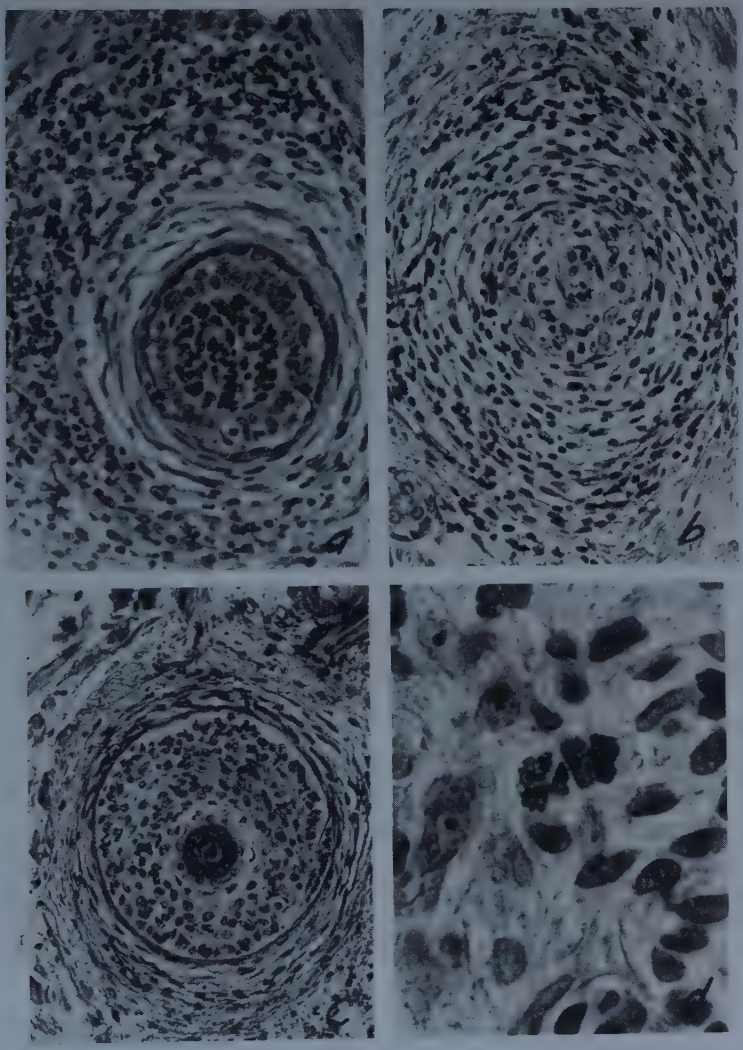

FIGURE 2. (*a*) Cellular infiltrate surrounding, but not invading, hair bulb. Bulb is anagen in type. ×260. (*b* and *c*) Hair roots at level above the hair bulb, showing that external sheath may or may not be invaded by inflammatory cells. (*d*) Mitotic cell in dermal hair papilla. Indicative of attempted regeneration of damaged papilla. ×800.

furthermore, they give indication of the general mechanism by which interference with growth of hair occurs in each instance. In alopecia areata essentially three categorical defects may be microscopically identified in roots of hair epilated from the margins of the balding areas.

Frequently found is the hair whose proximal end is tapered to a point (FIGURE 3*a*). The length of this taper, that is, the distance from the proximal tip of the hair to the point distally where the diameter of the hair is normal, is indicative of the time interval in which the hair follicle completed its transition from

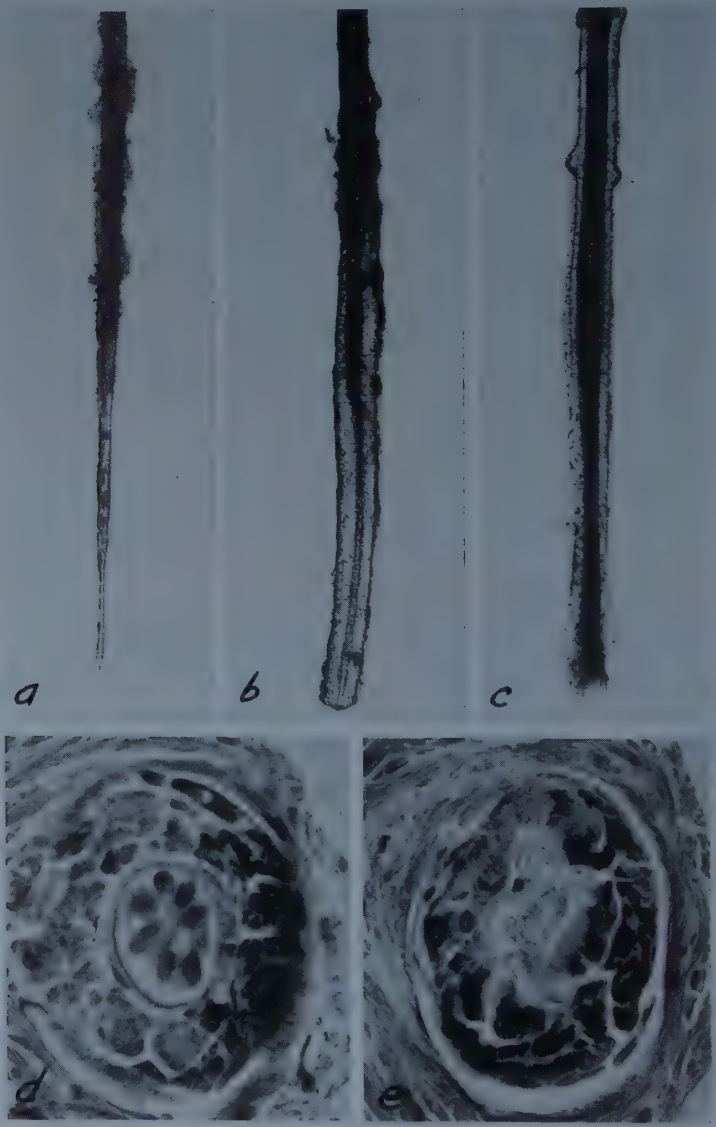

FIGURE 3. (*a*) Epilated hair with proximal (root end) portion tapered to a point. ×50. (*b*) Epilated hair. Proximal shaft is markedly diminished in diameter. Note that internal root sheath is present and normal in size. ×55. (*c*) Hair with zone of diminished diameter. Diameter at most proximal tip, immediately above hair bulb, is more normal. ×50. (*d*) Transverse section through rudimentary hair root of scalp that has grown no hair for 20 years. ×645. (*e*) Section beneath rudimentary hair root shown in *d*. Papilla cells absent. ×645.

normal production of a hair to complete interruption of hair production. Assuming a rate of growth of hair of approximately 0.35 mm. per day,[10] a taper 1 mm. long, for example, indicates that the follicle became completely abnormal in regard to hair production within a period of 72 hours.

Somewhat less frequently found is the hair, apparently produced by a follicle, in which there is incomplete disturbance of hair proliferation, with the proximal shaft markedly reduced in diameter but with no pointed tip (FIGURE 3*b*).

Third, there is the hair with a zone in its shaft diminished in diameter, but with its most proximal tip again approximately normal in diameter (FIGURE 3*c*). Such a hair would seem to result from a follicle in which production of a hair has been only temporarily disrupted, at least a partial recovery following.

Permanent baldness in alopecia areata. In some cases of alopecia areata all hair is lost from the entire body (alopecia universalis). Persistent baldness may result, although tufts of hair may temporarily regrow at varying intervals.[11] Cases occur only rarely in which absolute baldness of the scalp permanently persists. I observed such a patient, a 23-year-old man with absolute and persistent alopecia of the scalp persisting since he was 3 years old. Rudimentary hair roots, detectable as buds of epithelial cells extending downward from the base of the follicular neck, are histologically evident in specimens of scalp of this patient (FIGURE 3*d*). Dermal papillae are absent beneath such epithelial buds, however (FIGURE 3*e*).

Comparison of Alopecia Areata With Other Types of Alopecia

Male baldness. Baldness here is actually but an apparent absence of hair from the scalp resulting from the terminal hairs becoming progressively shorter in length and finer in calibre.[3] There is no loss of hair comparable to that in alopecia areata.

Inasmuch as the size of the hair matrix determines the size of the hair produced it is not suprising to find the volume of the matrix also reduced in the balding male scalp. Directly correlated with this reduced volume of the matrix however and, perhaps, causally related to it, is a proportionate decrease in volume of the dermal hair papilla.[3] This also is quite unlike the circumstance in alopecia areata, where the volume of the matrix undergoes a disproportionately greater decrease in comparison to the degree to which the volume of the papilla is reduced.

Whereas the hairs produced by the follicles in male baldness become smaller, the sebaceous glands do not become smaller but enlarge instead. Vellus hair follicles show no apparent changes in regard to either the size of hairs or of the sebaceous glands, and they may be distinguished from the terminal hair follicles by their smaller sebaceous glands as well as their more superficial location in the skin. Thus the balding scalp becomes much like the skin of "nonhairy" areas in that terminal hairs are small, but vellus hairs are still smaller.

The blood vessels of the ageing scalp become generally reduced in number, as demonstrated by Ellis.[12] No isolated changes in the blood supply of the dermal hair papilla in the balding male scalp have been recognized.

Alopecias resulting from infections of the scalp. Pyogenic infections of the scalp may cause loss of hair, but the mechanism of this would seem to be

mechanical, that is, actual destruction of follicles by enlarging abscesses. Permanent baldness may result from scar formation.

Some special attention should be directed toward alopecia occurring in secondary syphilis. This alopecia cannot be further investigated today because of its practical nonexistence, at least in most parts of the world. Alopecia occurs, however, when lesions of secondary syphilis involve the scalp, when spirochetes invade the skin. According to the histological description and photomicrographic presentation of the alopecic scalp by Wile and Belote in 1926,[13] an inflammatory cellular infiltrate surrounds the hair bulb but does not invade the bulb. This characteristic of the cellular infiltrate seems to be markedly similar to what is found in alopecia areata. These investigators did not specifically mention the state of the blood vessels supplying the hair papilla, but inasmuch as damage to the endothelium of blood vessels of the skin characteristically occurs in lesions of syphilis, it perhaps may be assumed that the same damage would be found in blood vessels supplying the hair papilla. The possibility that Wile and Belote described alopecia areata that by chance occurred simultaneously with secondary syphilis is rather remote in view of the fact that their patients promptly regrew hair following antiluetic therapy.

Alopecia following illness. This type of alopecia characteristically occurs 2 to 3 months following an episode of high fever and occurs because the majority of scalp hairs simultaneously enter the telogen phase of the growth cycle at the time of the febrile episode. The telogen phase of hair follicles of the average normal scalp may be mathematically estimated to last for approximately 3 months, assuming that the approximate average length of anagen in the scalp is 30 months and that an average of approximately 10 per cent of scalp hairs are normally telogen in type.[14] Therefore hairs entering the telogen phase during the febrile episode may be expected to fall from the scalp about three months later. Pinkus[15] has observed that shafts of hairs may be thinner than normal in postinfectious hair loss; I have not observed this defect in hairs following nonseptic fevers.

Loss of hair may also occur 2 to 3 months postpartum or in patients with severe debilitating diseases such as cancer. Similar to that occurring following fever, hair loss occurs because the majority of scalp hairs simultaneously entered telogen earlier.

In none of the above instances is hair loss permanent. Regrowth of hair promptly begins following the shedding of telogen hairs, as might be expected to occur normally.

Postradiation hair loss. Changes occurring in hair roots in response to ionizing radiation are characteristic for this injury. The histological changes in hair roots of the human scalp following epilating doses of X ray have been thoroughly described by Montagna and Chase.[16] The characteristic defects are visible in anagen hairs plucked from irradiated scalp.[17]

Within twenty-four hours following radiation mitotic activity in the matrix of the hair ceases. Thereafter the hair bulb, exclusive of the dermal papilla and the external root sheath, undergo progressive degeneration and disintegration. Melanin pigment, normally present only in the hair bulb and keratinized hair, migrates downward into the dermal papilla. Atrophy of the hair root

is complete within two to three weeks and hair falls from the scalp spontaneously at that time.

Most anagen hairs respond to radiation in all-or-none fashion, and any defect found in hairs plucked from irradiated scalp usually is the result of the above process of progressive degeneration. A few hair roots, however, may be found to recover from the radiation injury and not undergo degeneration. The hair shaft produced during this recovery period is thinner than normal and may be identified later as a constriction in the shaft of the hair. This zone of constriction of course moves distally as the hair continues to grow.

There is no evidence that hair roots are permanently destroyed with small doses of X ray, of 500 r or less. A new hair root is regenerated following loss of hair from this amount of radiation and regrowth of hair from previously damaged roots begins approximately 8 to 11 weeks following the radiation exposure. Growth of hair that is seen before this time is from follicular roots that were resting (telogen) at the time of radiation.[16]

There seems to be little comparison between the pathological changes in hair roots following radiation and the changes found in alopecia areata, aside from the fact that in both instances hair loss occurs and hair production is disturbed. No degeneration of hair roots occurs in alopecia areata that is at all similar to the degeneration following radiation. Hair roots in alopecia areata continually remain active and attempt to produce hair.

Hair loss following certain antimetabolic and antimitotic chemotherapeutic agents. Many new antimetabolic and antimitotic agents used in investigative chemotherapy of cancer, leukemia, and other diseases are found to cause hair loss in humans. Hair loss following folic acid antagonists (aminopterin, A-Methopterin, dichloroamethopterin), 5-fluorouracil, actinomycin D, and colcemid has been studied and found to be characteristic in type.[14,18] Whereas progressive degeneration of the hair root accounts for loss of hair following ionizing radiation, no such degeneration has been found in hair roots following systemic administration of these drugs. A temporary disruption of hair production occurs instead, even though mitotic activity in the hair matrix may be reduced to zero during this temporary period. Following this, mitotic figures reappear in the matrix, if therapy with the drug is stopped, and normal production of hair resumes. A constriction in the hair shaft, a zone wherein the diameter of the hair is diminished, appears later. The constriction moves distally as the hair continues to grow. Since this growth continues at a normal rate the distance of the constriction from the hair bulb is indicative of the elapsed time interval following drug therapy. Thus weeks and months after single doses of A-Methopterin or aminopterin have been given to patients I have been able to ascertain from microscopic examination of epilated scalp hairs the approximate date when the drug had been given by dividing 0.35 (average normal daily rate of growth of scalp hairs in millimeters) into the length (in millimeters) of hair between the constriction and the hair bulb. Hairs from the scalp of patients who have received several daily doses of A-Methopterin show a zone of constriction that is proportionally longer than that following a single dose. This zone may not be so easily visualized when doses have been low as the discrete zone of constriction following a single dose.

As in alopecia areata, mitotic figures and melanin granules appear in the dermal hair papillae of patients who have received A-Methopterin.

Comments and Interpretations

The findings in alopecia areata of degenerativelike changes in the connective tissue surrounding the blood vessels supplying dermal papillae, a dense cellular infiltrate subjacent to and surrounding the hair bulb, decrease in the volume of the hair papilla, and reduction in number of cells of the hair papilla suggest that impaired production of hair in this disease may be due to damage of the hair papilla that has resulted, in turn, from an interference with the blood supply of the papilla. This hypothesis would indicate then that the changes in the connective tissue surrounding the blood vessels supplying the dermal papilla may be considered as the primary lesion in alopecia areata.

From the apparent similarity between histopathological changes in alopecia areata with those in syphilitic alopecia the implication may be derived that the etiology of alopecia areata is also an infectious agent. Such speculative reasoning in regard to etiology is tenuous, however, in view of other abnormalities of alopecia areata that also occur in alopecias due to chemical or physical agents. An example of such an abnormality is the occurrence of melanin in the dermal hair papilla, which is found not only in alopecia areata but also following A-Methopterin and ionizing radiation. Migration of melanin from the matrix into the dermal papilla in all these instances would seem to be secondary to injury of the hair bulb and is comparable to epidermal loss of melanin following superficial cutaneous inflammation.

The conclusion that the dermal hair papilla is severely damaged in alopecia areata may be drawn from data showing that both its volume and cell population are markedly reduced. The presence of mitotic cells in the papilla in alopecia areata perhaps indicates attempted regeneration of the hair papilla.

Some comment should perhaps be made on the fact that hair roots in alopecia areata continually attempt to produce hair and do not enter a stage of involutional degeneration comparable to that following radiation. The preparedness of the hair root to produce hair in alopecia areata is evident from clinical regrowth of hair that can occur within one or two weeks following systemic therapy with adrenal corticoids. Hair roots in this disease are somewhat like hair roots affected by A-Methopterin, where the hair root is but temporarily restrained from producing a normal hair but promptly recovers following drug therapy and continues producing an apparently normal hair.

The volume, cell population, and contour of the normal hair matrix would seem to be maintained in a homeostatic state resulting from opposing dynamic forces within the matrix and outside of it. Continued production of cells by mitotic division within the matrix must create a degree of pressure that is directed outward in all directions; the connective tissue surrounding the hair bulb, in turn, must exert a constraining inwardly-directed pressure of a magnitude that prevents an ever-expanding enlargement of the matrix. A resultant directional force is created that moves the cells produced by the matrix into the follicular lumen for formation of the hair. Decrease in size and cell population of the matrix therefore results from a decrease in the outwardly-directed

pressure exerted by the matrix, due either to a decrease in the percentage of matrix cells undergoing mitotic division in a given period of time or to a prolongation of the mitotic interval between prophase and telophase. Since the volume and cell population of the hair matrix is reduced in alopecia areata, interference with mitosis of cells of the matrix by either or both of the foregoing mechanisms would seem to occur necessarily even though casual histological examination reveals the hair matrix in alopecia areata to be mitotically active. The presence of a normal or even greater than normal number of mitotic cells per volume of matrix in alopecia areata suggests that mitosis may be prolonged.

Possible modes of action of adrenal corticoids in restoring growth of hair in alopecia areata are consequently manifold. Whether these hormones ameliorate the changes in the connective tissue surrounding the blood vessels supplying the dermal papilla, whether by an anti-inflammatory action they diminish the cellular infiltrate surrounding the hair roots, or whether they more directly stimulate hair production (as they stimulate growth of the rat's tooth[19]) are questions that cannot be resolved from present data.

Summary

The disturbed growth of hair in alopecia areata is reviewed in light of present knowledge of normal hair growth and is compared to different types of disturbance of hair growth in other pathological conditions. Possible mechanisms of the interference with growth of hair in alopecia areata, interpreted from pathoanatomical and pathophysiological findings, are considered.

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Part IV. Neogenesis of Hair Follicles in Adult Skin

THE NEOGENESIS OF SKIN IN THE ANTLERS OF DEER*

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Introduction

To most biologists it is almost if not quite axiomatic that adult mammals cannot regenerate normal skin in areas where its entire thickness has been lost or destroyed. The very existence of a specialized branch of surgery that deals largely with the making good of cutaneous lesions is testimony of the incompetence of man to generate new skin.

The natural healing of a wound involving the loss of the full thickness of the skin is the result of two more or less concomitant processes. First, the loss of the substance of the corium is made good by the formation of highly vascular granulation tissue that is progressively resurfaced by epithelium of migratory origin from the wound margins. Second, as a result of tensile forces generated within the wound area itself, and almost certainly within the substance of the granulation tissue or its derivatives, the normal skin from the original wound margins becomes drawn inward, and there is a consequent reduction in the extent of the lesion (Billingham and Medawar, 1955; Abercrombie *et al.*, 1956; Billingham and Russell, 1956*a*; Watts *et al.*, 1958).

In regions of the body where the skin is loosely knit to the underlying structures and is therefore freely mobile, as in the trunk of nearly all mammals, contracture usually brings about complete closure of the wound by apposition of its original edges, so that only a narrow scar remains. The loss of skin substance that this process entails is gradually made good by a compensatory expansion of the surrounding skin by a process of intussusceptive growth—that is, the formation of new tissue upon or within the framework provided by pre-existing tissue (Billingham and Medawar, 1955; James, 1959). However, in areas of the body where the skin is firmly attached to the underlying tissues (as in the skin over most of the body of man and in the skin covering the ear cartilage of most animals) contracture cannot proceed to completion. There is a permanent residual scar of variable extent, comprising tough, relatively inelastic, white fibrous tissue covered by epithelium. The formation of this stable scar cannot be regarded as a regeneration since nothing has been regenerated: scar tissue is certainly not true skin that has been formed anew. It differs profoundly from normal skin with respect to the configuration of its dermoepidermal interface and its fibrous architecture. There is a deficiency of elastic fibers and a tendency for its collagen fibers to be disposed in a horizontal plane rather than in the usual three-dimensional packing. This is responsible for its poorly developed resilience. Hairs are not normally regenerated anew, although it is commonly said that regeneration of sweat glands may take place in scar tissue in man.

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Partly because of the widespread belief that adult mammals cannot regenerate normal skin anew, and partly because of an equally widespread view—indeed, it is almost an embryological dogma—that the primordia of all hair follicles of mammals are laid down before or soon after birth, nearly all claims to have observed or demonstrated hair neogenesis in adult animals have been received with considerable skepticism. Although many of these claims are ill-founded, it has been established beyond all reasonable doubt that if contracture of cutaneous wounds in the trunk skin of rabbits is prevented by appropriate experimental artifice, then hair neogenesis does take place in the scar tissue on a considerable scale (Breedis, 1954; Billingham and Russell, 1956*b*; Billingham, 1958).

The purpose of this article is to give an account of a phenomenon that has a decisive bearing upon the fundamental question of whether the neogenesis of skin itself, complete with its appendages, can or ever does occur.

The Growth and Replacement of Antlers in Deer

An almost diagnostic characteristic of the Cervidae, or deer, is the development of antlers. As a rule these are present only in the males, although in one or two species, such as the reindeer or caribou (*Rangifer tarandus*), they are also present in the females. Antlers are deciduous bony growths that arise from permanent, short, bony pedicles on the frontal bones of the skull. They are shed annually and, subsequently, they are regenerated with progressively more numerous lateral branches, or tines, as the animals grow older. This cycle of growth and replacement is under endocrine control and has been studied in considerable detail by the late G. B. Wislocki and his associates at Harvard University, Cambridge, Mass. For us, the particular importance of deer antlers lies in the fact that throughout their development, and until they have attained maturity, they are completely covered by a layer of hair-bearing skin, the "velvet."

In the white-tailed or Virginia deer (*Odocoileus virginianus*) familiar in the eastern part of the United States, growth of the antlers begins in April or May and is completed in August. Soon after cessation of antler growth the velvet becomes necrotic, probably as a consequence of obstruction of its blood circulation as ossification of the peripheral zone of the antler shaft proceeds (Waldo *et al.*, 1949; Wislocki, 1956). The dry velvet soon peels away from the mature antlers. The antlers, now consisting of bare dead bone, remain firmly attached until midwinter. From their extensive investigations, Wislocki *et al.* (see Wislocki, 1956) conclude that the eventual separation and shedding of the antlers from their basal pedicles is due to proliferation of connective tissue that accompanies the resorption of the osseous connections.

After the antlers are shed, the pedicles are evident as short, bony elevations covered laterally with a continuation of the skin of the forehead, which bears a dense crop of coarse hairs. At the apex of each pedicle is a slight concavity from which the proximal end of the antler has become disengaged. This concavity becomes filled with fibrocellular granulation tissue that is soon resurfaced by inward migration of epithelium from the "wound" margins (Macewen, 1920; Wislocki, 1942). At first this apical zone remains smooth

and bald, but soon soft, downy hairs arise from it. After a few weeks, growth of the antlers begins and proceeds at a phenomenal rate through the continuous proliferative activity of cells in the germinal or apical regions of the pedicles and the consequent laying down of new material at the extremities. Daily increases in length of the order of 1.2 to 1.5 cm. have been recorded in the growing antlers of elk (*Alces machilis*) and caribou (Wislocki and Singer, 1946). Thus the apical growing points are borne outward from the antler pedicles on the column of bone that they leave in their wake. This bone remains more or less unaltered in diameter as it matures. As the main beams elongate, new apical regions arise laterally and so give rise to the tines or lateral processes.

FIGURE 1. Head of an elk bearing enormous palmate antlers. (W. H. Flower and R. Lydekker, 1891.)

Thus all stages of growth and maturation are present in a growing antler. In some species such as the elk the entire antler assumes a palmate or frondlike shape (FIGURE 1). The size of the mature antlers varies widely according to the species. In the now extinct Gigantic Irish Elk (*Cervus giganteus*) the enormous palmate antlers had a span of more than 11 feet and are said to have weighed more than the remainder of the skeleton. The enormous palmate antlers of the elk may weigh as much as 60 lb.

The Velvet

The velvet, whose growth keeps pace *pari passu* with that of the osseous structure, is directly and very firmly united to the periosteum of the latter. The usual fat-containing subcutaneous layer of loose connective tissue is absent (because of this, infiltration of the velvet with solutions of local anesthetics is very difficult to achieve.) The epidermis of the velvet is usually

much thicker than that of the general integument (FIGURES 2, *upper and lower*, and 3). Unlike the latter, it is very deeply pigmented as a consequence of the abundance and activity of melanocytes in its basal layer. The *entire* velvet is covered by a fairly dense crop of short, soft, pigmented hairs that tend to emerge at right angles to the surface and are more sparsely distributed and more delicate at the apical regions than elsewhere on the antlers. Associated with these hairs are well-developed sebaceous glands. Like Wislocki and Singer (1946), we

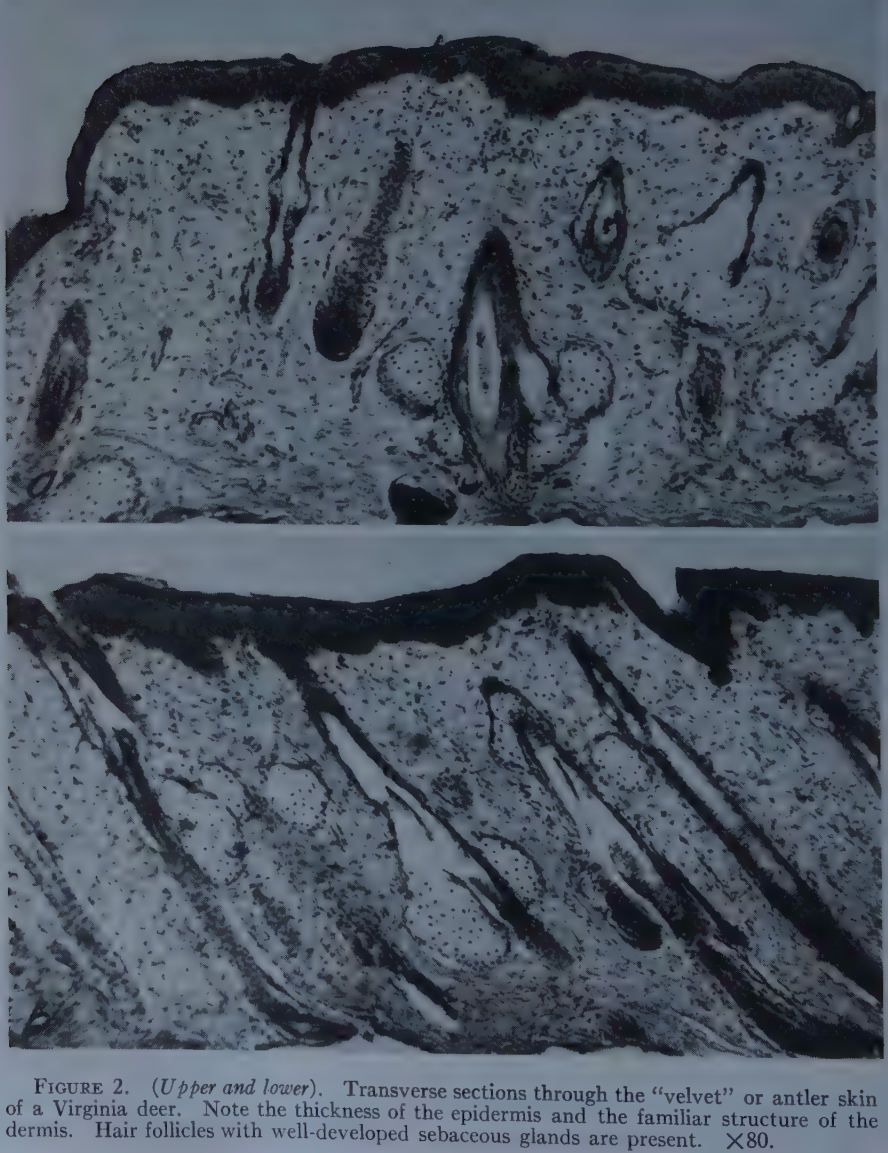

FIGURE 2. (*Upper and lower*). Transverse sections through the "velvet" or antler skin of a Virginia deer. Note the thickness of the epidermis and the familiar structure of the dermis. Hair follicles with well-developed sebaceous glands are present. ×80.

have been unable to find arrectores pilorum muscles in association with the pilosebaceous units of the velvet. In this respect, therefore, the hairs of the velvet resemble those which form *de novo* in incompletely contracted wounds in the skin of the rabbit (see Billingham and Russell, 1956b). Although sweat glands are present in the skin of much of the body of the Virginia deer, they are absent in the velvet. According to Vacek (1955), however, they are present in the velvet of stags (*Cervus elaphus*) and of fallow bucks (*Dama dama* [see Lojda, 1956]). The corium of the velvet does not differ significantly from the

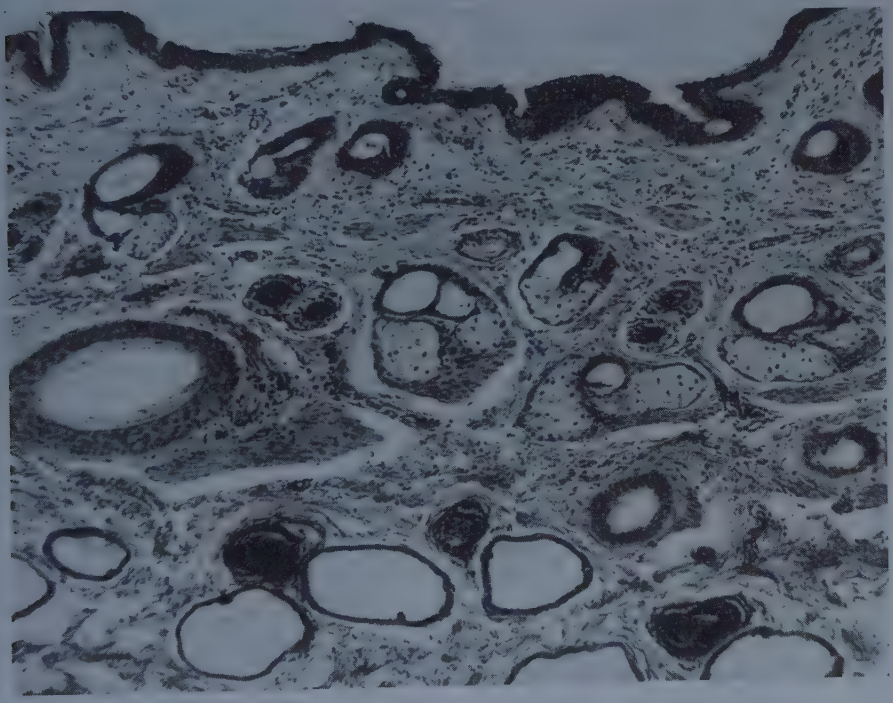

FIGURE 3. Transverse section through normal body skin (from hind limb) of a Virginia deer. Note that the epidermis is much thinner than that of the velvet; the dermis is much thicker and, in addition to the pilosebaceous units, contains abundant apocrine glands. ×80.

skin elsewhere on the body (FIGURES 2, *upper and lower*, and 3). Its outer layer gives rise to papillae that penetrate the epidermis. The relatively thick reticular layer, which contains the pilosebaceous units, is firmly united to a collagenous tunic, in which the main blood vessels supplying the antler are located. Because of the physical inseparability of this vascular layer from the overlying skin, Wislocki (1942) regarded it as a highly modified subcutaneous lamina, and not as the outer layer of the periosteum of the antler to which it is firmly adherent.

Although evidence of sensory perception in growing antlers has been presented and abundant nerve fibers are demonstrable in the vascular layer of the antler, specialized nerve endings have not been observed in the corium of

the velvet. Accordingly, the suggestion has been put forward that the nerve fibers in the skin terminate in simple endings (Wislocki and Singer, 1946). The growth of the nerves certainly keeps pace with that of the developing antlers.

Discussion and Conclusions

From this brief account of its structure it will be apparent that the velvet is normal cervine skin in all respects, as previous investigators have emphasized repeatedly (Macewen, 1920; Noback and Modell, 1928; Lojda, 1956). The velvet certainly bears not even the most superficial resemblance to scar tissue.

The sheer magnitude of the antlers in some of the larger species of deer renders completely futile any attempt to explain the origin of the velvet, with its appendages, in terms of any process other than that of neogenesis from the cellular elements present in the pedicles. It is exceedingly difficult to attribute it to intussusceptive growth as Lojda (1956) has done. Unfortunately, at present we know very little about the cellular processes that underlie the morphogenesis of antlers. Macewen (1920) very appropriately drew attention to the analogy that exists between the regeneration of an antler and the regeneration of limbs or tails in certain lower vertebrates. The germinal caps of the antlers bear a striking resemblance to the blastemas from which amputated limbs regenerate.

Although the velvet is normally ephemeral, it is perfectly capable of indefinite survival provided that its blood supply is assured. Castration of deer after the appearance of the first set of antlers, at a time when the latter are still in the velvet, leads to permanent retention of both antlers and their velvet (see Wislocki, 1956). Furthermore, as we have found, if grafts of the velvet are transplanted elsewhere on the body of a deer—for example, to the skin of the hindlimbs—they survive long after the antlers have been shed. Studies on velvet that is enabled to persist indefinitely by either of these two methods should provide an answer to the question of whether hair succession occurs.

Our preliminary studies on the healing of full-thickness wounds made in the skin overlying the metatarsal bone of the hindlimb—to which it is rather firmly united—indicate that healing takes place as usual by the formation of scar tissue that remains hairless. It is probable, therefore, that the capacity of a deer to form new skin is strictly localized to one region of its body—over the pedicles of the frontal bones of the skull. It will be interesting, however, to study the repair process in wounds inflicted in the living velvet of growing antlers.

Although it was Wislocki's (1942) opinion that growth of antlers is probably dependent upon, and controlled by, the formation of a germinal or periosteal bed derived from the skin, the nature of the interaction between the cutaneous and bony rudiments that results in antler growth is still a completely open and provocative question. Jaczewski's (1956*a* and *b*) successful free transplantation of an antler rudiment to a more anterior site on the frontal bone of the skull of a red deer stag, where it gave rise to an antler, and his finding that an antler was regenerated on the donor site of the graft, indicate that the problem is amenable to experimental analysis. We are hoping to replace the skin covering the antler rudiments in young Virginia deer stag fawns with skin

from other regions of the body—for example, the outer aspect of the ear—to see whether there is any evidence of regional competence on the part of the integument to participate in the formation of antlers.

Summary

So firmly established is the concept that normal skin is not regenerated anew in healing wounds in adult mammals that any claims to have demonstrated the neogenesis of even some of the appendages of skin, particularly hairs, in scar tissue usually are received with grave suspicion.

Fortunately, the principle that at least some adult mammals are capable of hair neogenesis, and even of skin neogenesis, does not have to rest on experimental evidence derived from studies on small cutaneous lesions. The antlers of deer are deciduous, bony outgrowths from pedicles on the frontal bones of the skull. Throughout their growth, which occurs at a phenomenal rate, antlers are covered by a layer of normal cervine skin, complete with pigmented hairs and glands. This layer of skin, usually known as the velvet, is normally shed after growth of the antlers is complete. The antlers themselves are not shed until midwinter. Then, the following spring, the antlers are generated anew from the pedicles. This annual event continues throughout the life of the animals. Here, then, is an unequivocal example, not only of the neogenesis of hair, but of skin itself, performed on the grand scale without the necessity for experimental artifice. No other explanation can conceivably account for the formation of extensive areas of velvet each year than that of complete neogenesis.

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A STUDY ON THE NEOFORMATION OF MAMMALIAN HAIR FOLLICLES*

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Introduction

Until recently, there were few reports in the literature of a neoformation of hair follicles in adult mammalian skin. Lacassagne and Latarjet (1946) described the growth of new hair follicles and sebaceous glands in the scars of burns produced by ultraviolet rays in the skin of mice. Noticing that short, fine hairs appeared on scars of healed wounds in rats, Taylor (1949) stated that "... the size of the hairs and the time of their appearance strongly suggest that there is also a differentiation of new follicles on the regenerated skin." A contrary opinion was expressed by Wolbach (1951) who believed that an "Increase in the number of hair follicles, that is, new formation from the epidermis does not take place in the mouse."

A resurgence of interest in the neoformation of mammalian hair follicles has recently occurred (for example Gillman *et al*, 1955; Orr, 1955; Billingham and Russell, 1956; Kligman and Strauss, 1956), but few recent contributions to this subject have received as much notice as that of Breedis (1954). In the depilated skin of rabbits he made circular wounds, 25 mm. in diameter and involving the entire depth, and enclosed them in stainless steel chambers covered with glass. Breedis found that this procedure prevented the drying and contraction of the wounds, two factors that he said were essential for the neoformation of hair follicles in wounds. From whole mounts of split epidermis, taken from the healing wounds at various intervals of time, Breedis observed that buds of epidermal cells formed along ridges of epidermis at the periphery of the wounds and later in the central region. New hair follicles gradually developed from these buds. Sebaceous glands differentiated when the follicles began to produce hair. Although Breedis believed that this was a neoformation of hair follicles from the wound epidermis, he recognized the possibility that fragments of follicles from the wound margin could have migrated with the epidermis and later regenerated into whole follicles.

In the present study, the movements of hair follicles were followed at the border of wounds that were healing within chambers similar to those devised by Breedis. The results indicated that the chambers did not prevent wound contraction because uninjured follicles moved from the periphery into the wounds and repopulated them without evidence of a neoformation.

Materials and Methods

Twelve adult black and dilute black Dutch rabbits were used in this experiment. Chambers were constructed (FIGURE 1) like those designed by Breedis

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(1954). Each chamber consisted of three parts: a wire ring of 22-gauge, surgical, stainless steel wire; a stainless steel chamber 33 mm. in diameter; and a watch-glass. The steel chamber had 3 projecting lugs, which facilitated the attachment of the watch glass with 25-gauge, stainless steel wire.

In each animal the hairs were plucked from pigmented resting follicles lateral to the mid-dorsal line to initiate a new cycle of hair growth (David, 1934). A circular area of skin 70 mm. in diameter was X-irradiated in the plucked area, but a central area 35 mm. in diameter was protected with a circular lead shield. A dose of 1000 r at 1000 r/min. was given without filters from an X-ray therapy machine operated at 200 kV and 20 ma. at a target-skin distance of 17 cm. The pigment cells in the irradiated follicles were destroyed (Danneel and Lubnow, 1936; Chase, 1949), and white hairs subsequently emerged from them (FIGURE 2). When these follicles were again in the resting stage, their

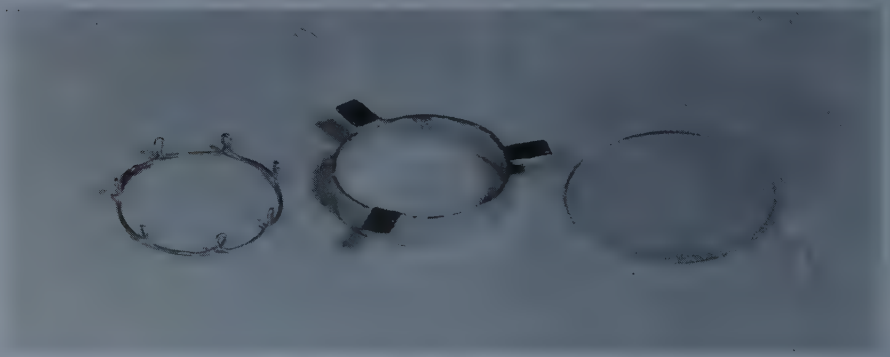

FIGURE 1. At left is the wire ring that was positioned beneath the skin. In the center is the stainless steel chamber with projecting lugs for the attachment of an ordinary watch-glass, shown on the right.

club hairs were plucked out once more. Ten days later the animals were wounded. At this time the circular shielded area of skin was dark gray because of the developing pigmented hairs; but the surrounding irradiated skin, where white hairs were forming, remained pink (FIGURE 3).

Before wounding, the entire area was swabbed first with a solution of iodine in ethanol and then with ethoxyethane. A 25-mm. diameter area of skin including the cutis muscle was aseptically removed from the center of the circular 35-mm. nonirradiated area. The wound was thus surrounded by a band of black hairs 5 mm. wide (FIGURE 4). Seven small puncture wounds were made at equally spaced intervals around the large wound. The wire ring was placed beneath the 25-mm. diameter wound, and its wire loops were inserted through the 7 puncture wounds. The stainless steel chamber was then fastened with 28-gauge wire to the prongs, which extended above the skin; and the watch glass was attached. A few days later, when a scab had formed, petrolatum and gauze dressings were placed over the wound within the chamber; and they were changed every 4 days until 49 to 76 days after wounding.

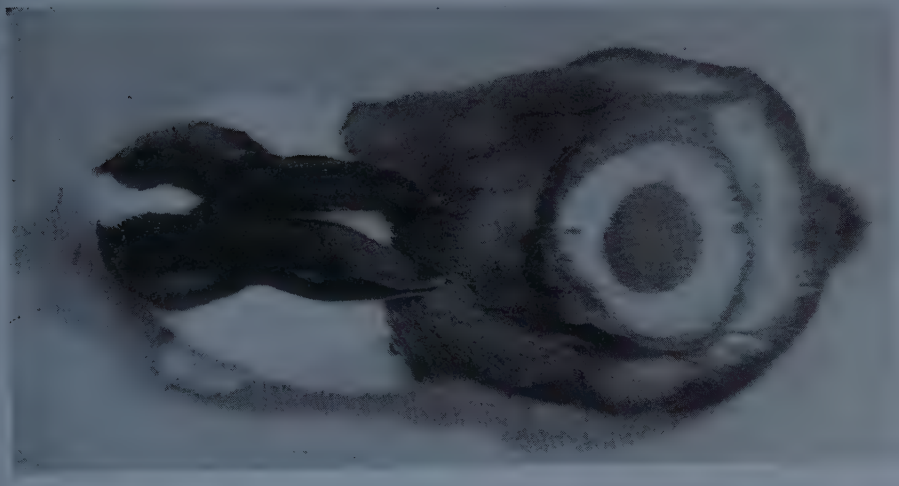

FIGURE 2. Notice the predominance of white hairs in the X-irradiated region. Plucking before irradiation had initiated a new cycle of hair growth.

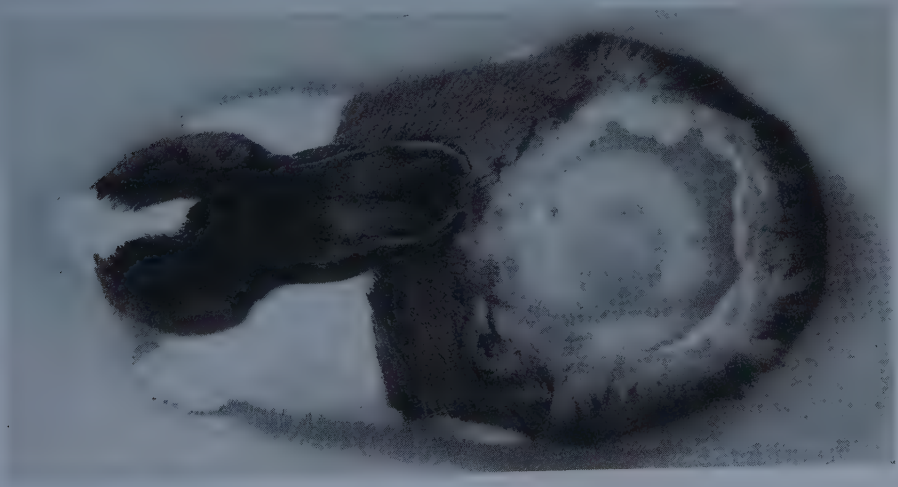

FIGURE 3. This bare area of skin was plucked 11 days before the photograph was taken. The subsequent new growth of hairs had not erupted from the skin; however, the shielded circle of skin was clearly delineated by the color of its developing hairs.

Results

In the 10 wounds that did not become infected, scabs started to form at 8 to 21 days and they separated by 28 to 37 days. The time of scab formation and separation was delayed in the two infected wounds; otherwise, the subsequent events were similar. Before the separation of the scab or shortly thereafter, growing follicles with pigmented hairs began to move centripetally into each wound. Regardless of the restraining effect of the chamber, the skin

was contracting. Many of the follicles along the borders of the wounds were displaced. The bulbs of these follicles were often shifted toward the centers of the wounds.

By 45 days after wounding, many of the club hairs of the resting follicles within the chambers had fallen out; and the skin within the chambers often showed pronounced inflammation and edema with an absence of hair growth. These effects were due to the petrolatum that constantly covered the area; when the petrolatum was no longer applied, the inflammation subsided and hair growth started.

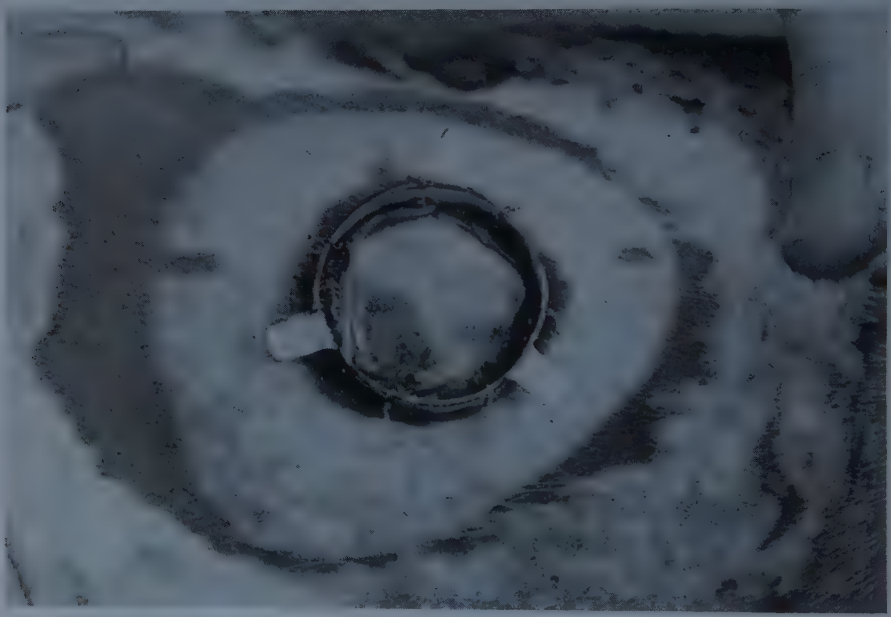

FIGURE 4. Photograph taken 17 days after plucking and 6 days after the chamber was attached. Observe the position of the pigmented band of hairs surrounding the wound. Because of wound retraction, the pigmented band is slightly displaced.

Within the chambers, at the termination of wound contraction there were small hairless scars, with approximate dimensions of 5 by 13 mm.; in all cases the long axes of these scars were perpendicular to the mid-dorsal axis of the body. Therefore, wound contraction occurred primarily in an anterior-posterior direction. The 5-mm. wide band of black hairs previously at the border of the wound had redistributed itself within the wound and surrounded the hairless scars in all 12 animals (FIGURE 5). The wound area was clearly repopulated by hairs from the periphery. At wounding, the wire prongs that pierced the skin had been within the black band of hairs; but after wound contraction most of the prongs were surrounded by white hairs (FIGURE 5). Occasionally, the white hairs even penetrated the 25-mm. diameter central area of the chambers (FIGURE 5).

After the scar was formed and wound contraction completed, localized infections appeared around some of the 7 wire prongs that penetrated the skin. These infections led to the formation of small scabs around and beneath the wire prongs. The skin then healed under the scab and, consequently, under the wire ring. The healing of successive infections resulted in a progressive extrusion of the ring (FIGURE 5).

FIGURE 5. Photograph taken 86 days postwounding. Notice the position of the black hairs which were previously at the border of the 25-mm. diameter wound. Observe the extreme centripetal displacement of the field of white hairs, which had moved past the prongs of the subdermal wire ring. Extrusion of this ring did not start until after the migration of hair follicles was completed.

Discussion

According to Breedis (1954), an essential condition for the neoformation of hair follicles in wounds is the prevention of wound contraction. Breedis designed the stainless steel and glass chambers for this purpose. In the present study similarly constructed chambers did not effectively impede the inward movement of skin because hair follicles at the borders of the wounds moved past the wire prongs into the central area of the chambers.

Hair growth was initiated in the present experiments by plucking out the club hairs several days before wounding. Since Breedis did not control the hair growth cycle, the skin of his experimental animals probably contained resting hair follicles because the resting phase in the skin of the rabbit is much longer than the growing phase (Rony *et al.*, 1953). The injury incurred during wounding would normally initiate the growth of nearby resting hair

follicles after a latent period of 6 to 8 days (Rony *et al.*, 1953), but Breedis did not indicate how the growth of hair was affected by his experiments. As it did in the present study, the adverse influence of petrolatum may have delayed the activation of the resting follicles near the wounds. Depilated follicles entering the growth phase, while being pulled into the wounds with the contracting skin, would resemble the newly formed follicles described by Breedis because the morphological changes during the regenerative phase of the normal hair growth cycle are largely identical to the pattern of hair follicle embryology (Chase *et al*, 1951; Chase, 1954). In the present experiments the inward movement of hair follicles, first noticed at 28 to 37 days postwounding, corresponded in time to the appearance of new hair follicles in the experiments of Breedis, who first observed a neoformation at 30 days after wounding. Breedis saw new follicles forming initially at the periphery of the wounds and later in the central region. This is consistent with the view that the follicles were actually being pulled into the wound with the contracting skin.

Billingham and Russell (1956) also reported a neoformation of hair follicles in healing wounds in rabbit skin. In some respects their description was similar to that of Breedis (1954). New hairs first appeared on bald areas of skin at the periphery of incompletely contracted wounds that had been dressed, presumably with petrolatum. These new hair follicles were displaced because their bulbs were shifted toward the centers of the wounds in the same manner as the contracting hair follicles in the present experiments. The bald, edematous, and slightly inflamed condition of the skin in the experiments of Billingham and Russell was similar to the state of the skin within the chambers in the present experiments after the club hairs had fallen out in response to the prolonged presence of petrolatum.

A loss of hair not only occurs near wounds that are dressed with petrolatum but also around large, undressed contracting wounds in the guinea pig (Straile, 1958). This was observed when the borders of untreated wounds were marked by tattooing. Additionally, hair loss was commonly observed in experiments involving a study of 7-mm. wide strips of skin between two wounds. These semi-isolated areas often became devoid of hair during wound contraction. When wounds are experimentally treated, hair loss may be more severe and may mask wound contraction, which is difficult to observe when hairs are absent.

An abundant neoformation of hair follicles does take place annually during the development of cervine antlers (Billingham, 1958). With this in mind, one cannot say without reservation that hair follicle neoformation does not take place in the skin of adult mammals. The purpose of the present study is not to disprove the prevalent idea that hair follicle neoformation additionally takes place in wounds in adult mammalian skin, but to point out the need for more decisively designed experiments on this subject.

When mammalian skin moves into wounds it is labile and can undergo substantial adjustments in surface area without any significant change in the quantity of its collagen (Straile, 1958). The present study, which shows that rabbit skin can be pulled around metal barriers, emphasizes the great plasticity of the framework of dermal collagen fibers in skin near contracting wounds.

Summary

Stainless steel chambers similar to those designed by Breedis (1954) to prevent wound contraction were ineffective under the conditions described in this paper.

Hair follicles at the periphery of wounds healing within these chambers converged centripetally and repopulated the entire wound areas except for small centrally placed scars, which remained hairless. Evidence for a neoformation of hair follicles was lacking.

The presence of seven stainless steel prongs, piercing the skin at equally spaced points along the circumference of each chamber, did not significantly impede the inward movements of the dermis and hair follicles. Therefore, the framework of dermal collagen fibers in the skin near the contracting wounds must have been very plastic and capable of considerable rearrangement in response to the tensions that were generated in the contracting wounds.

The use of petrolatum in the wound dressings caused a pronounced inflammation in the adjacent skin and a subsequent loss of club hairs without a normal regrowth. The later reappearance of hair on these bald areas of inflamed skin incorrectly resembled a neoformation of hair follicles.

Histological and morphological studies on the neoformation of hair follicles in wounds should be supplemented with techniques that allow the investigator to accurately follow the movements of the hair follicles which are adjacent to the wounds.

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NEOGENESIS OF HUMAN HAIR FOLLICLES*

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The half life of scientific ideas is approximately the interval between meetings. This period is contracting rapidly. The ancient dispute concerning mammalian hair neogenesis was seemingly settled by Breedis' ingenious demonstration of hair regeneration in full-thickness rabbit wounds that had been prevented from contracting.[1] If further proof was needed it was generously supplied by Billingham and Russell's observation of the formation of new follicles from the epidermis of incompletely contracted rabbit wounds.[2] Today, the workers at Brown University reopen the question by pointing out an occult pitfall that Breedis did not consider. Almost as interesting as the fresh doubts they have raised is the reason why they continued to be skeptical in the face of such imposing evidence. Our "proof" of human hair neogenesis will probably fare a little better only because it is not legally possible for the workers at Brown University to repeat the experiments.

In humans, as in lower animals, the complement of hair follicles is not normally increased after a certain period in embryonic life. Quite a different problem is whether hair follicles can be regenerated after their destruction or removal. Man has an enormous psychological interest in hair; the fact that generations of observers have not witnessed regrowth of hair in scores of destructive diseases and traumas must mean that such a phenomenon, if it occurs at all, must do so under quite unique circumstances. This is indeed the case.

The known human instances of this phenomenon are of two kinds: that following dermabrasion[3] and, in certain pathologic states, chiefly benign neoplasms.

Neogenesis Following Dermabrasion

Dermabrasion is a technique extensively used to improve the facial scars left by inflammatory acne. The skin is frozen with a volatile refrigerant such as ethyl chloride and then planed with a rotating brush or wheel, removing the epidermis and the upper portion of the dermis. With deep freezing and vigorous effort, about 2 mm. of facial skin can be removed, almost halfway through the corium. Resurfacing with a fresh epidermis over a highly vascularized granulation tissue bed is complete in about a week. The details of wound repair are not inique and follow well-known laws, except for the highly agreeable property that there is no scarring.[4]

It is necessary to appreciate the anatomical peculiarities of the face. The follicles are of two sorts: a more numerous superficial set, similar to vellus follicles elsewhere, generally not greater than 1 mm. in length (FIGURE 1). These can be removed by vigorous dermabrasion. The orifices of these superficial follicles and the hairs they bear are not visible to the untrained observer except with magnification. The easily visible pores of the face are the openings of a

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special kind of follicle, the sebaceous follicle. It is so called because of its extravagantly lobulated and large sebaceous glands. The hair in such follicles is rudimentary and easily overlooked. Only the upper portion of these large sebaceous follicles is removed by dermabrasion and, consequently, re-formation of these follicles is a process of repair, not neogenesis. The following remarks apply to the regeneration of the superficial vellus follicles in five volunteers in whom biopsy immediately after dermabrasion verified the complete removal of vellus hair follicles. The detailed findings have been reported elsewhere.[3]

FIGURE 1. This section contains two large sebaceous follicles and four superficial vellus hair follicles. The tissue above the dotted line is removable by vigorous dermabrasion. Hematoxylin-eosin. ×42.

By two weeks solid epithelial cords, regularly spaced, have begun to grow down from the epidermis. Papillae were not clearly discernible as such but it must be remembered that the upper dermis was fresh granulation tissue, inflamed from the freezing and mechanical trauma. Except for the absence of clear-cut papillae these down growths resembled the primary epithelial germs of fetal life. By the third week, connective tissue had clearly invaginated the lower portion of the epithelial cords. It was only by its invaginated location in the hair bulb that the papilla could be recognized as such for at this stage it was far less cellular than normal. Sebaceous gland anlage were already present as rounded swellings of undifferentiated epithelial cells. True differentiation of the bulb into a keratin synthesizing structure was first in evidence at four weeks with the formation of a cone of internal root sheath

cells. Concomitantly the sebaceous anlage began to differentiate into fat laden cells. The pattern of development into a mature pilosebaceous unit was in no wise different from the embryonal scheme. By two months regeneration of the pilosebaceous unit was complete (FIGURE 2). All of these follicles were in anagen, implementing the conclusion that they were newly formed. Normally 50 per cent or more of vellus hairs are found to be in telogen, owing to their short growth periods.

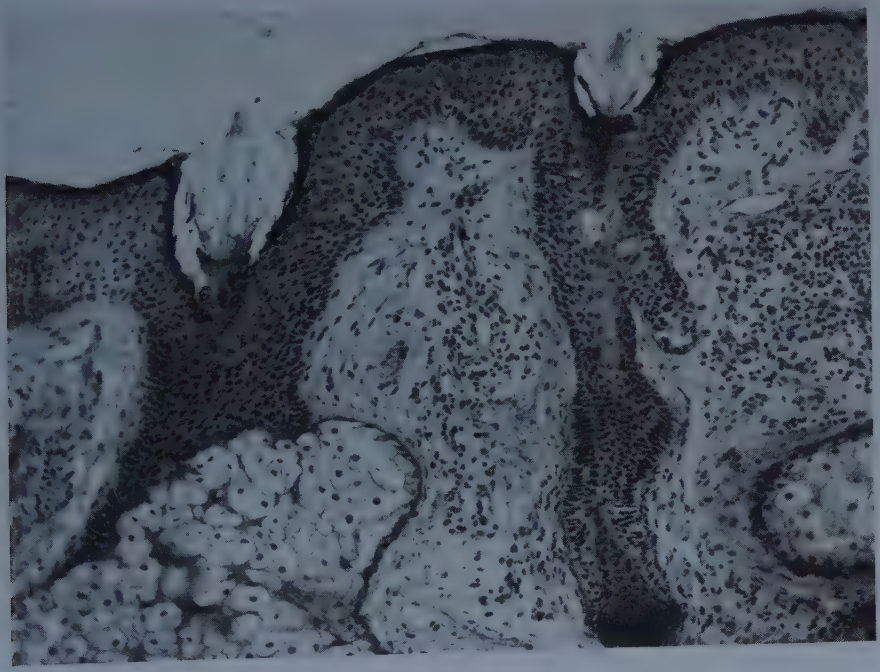

FIGURE 2. On the right is a regenerated vellus follicle 6 weeks after dermabrasion. The matrix has differentiated a cone of internal root sheath cells below. On the left is the upper portion of a repaired sebaceous follicle. The tissue is still inflamed. Hematoxylin-eosin. ×40.

Neogenesis in Pathological States

There is a diversified class of benign cutaneous tumors whose unifying quality is the tendency to form adnexal structures, that is, hair, sebaceous glands, and apocrine and eccrine glands.

These tumors grade all the way from more or less perfectly structured adnexal organs, down through moderately identifiable organs, to more or less completely undifferentiated cell masses.

Relevant to our topic is the best known of those that differentiate toward hair, the trichoepithelioma. Although the resemblance to hair follicle formation is generally exaggerated in textbook accounts, there can be no doubt that rudimentary follicles with recognizable papillae and bulbs are occasionally found. In rare instances differentiation may proceed to the genuine formation

of a keratinized hair shaft, albeit a feeble one. A recent analysis of this subject is given by Swerdlow.[5]

More to the point is a little known, probably rare, rather insignificant tumor in which differentiation toward hair follicles is a dominant, unmistakable feature. All degrees of organizational completeness are found ranging from embryonal-like epithelial germs, with and without papillae, through stages of incomplete anagen and, finally, to well-formed, fully competent hair-bearing follicles. Such tumors have been described by Fessler,[6] Prinz,[7] and Hyman

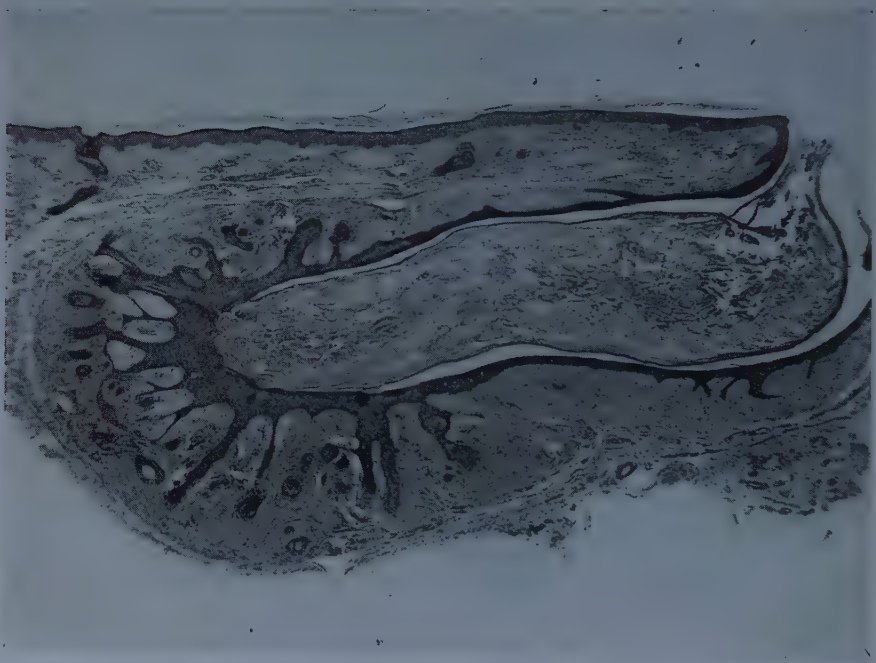

FIGURE 3. General architecture of the folliculoma. A distorted horn-filled follicle sends out epithelial radiations that form vellus follicles to varying degrees of completeness. Some are producing tiny, for the most part, imperfect hairs.

and Clayman[8] under the name of hair follicle nevus. The cases Pinkus and I are collecting to describe in print will be submitted under the title of folliculoma. The histopathological picture is stunningly pathognomonic (FIGURE 3). There is a central keratin-filled sinus or invagination lined with a highly unstable, proliferating epithelium that sends out anastomosing sheets and cords. The sinus is obviously a grotesque follicle. Many of the cords radiating from this sinus are well-defined hair-bearing follicles while others are incompletely differentiated pilary structures. Secondary and even tertiary clusters of these pilary constellations originate from the epithelial proliferations. Dendritic strands of undifferentiated cells link the follicles together so that the whole tumor is actually a network. Of incidental interest is the rich connective tissue stroma in which the pilary complexes are imbedded.

Comment

It is necessary to emphasize that the instances of neogenesis given here involve the formation of only diminutive, vestigial vellus follicles. The regeneration of a human terminal follicle bearing a hair worthy of the name will be a truly momentous finding.*

Summary

Neogenesis of human vellus hair follicles has been observed in facial skin following dermabrasion and in certain benign skin tumors.

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* Very recently Paul Gross and I have observed regeneration of moderately-sized follicles in scalp scars left after excision of the top 1 to 2 mm. of skin.

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